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LOW TEMPERATURE BREAKDOWN OF POTATOES IN STORAGE[1]

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An internal browning has frequently been observed in potato tubers, particularly of the Katahdin variety, in commercial warehouses. In order to determine whether or not this injury was the result of storage at low temperature, a joint investigation was begun in 1941 by the Division of Botany and Plant Pathology and the Division of Horticulture, Central Experimental Farm, Ottawa. It was found that tubers of certain varieties when stored for two months or longer at temperatures just above the freezing point of the tissues developed a physiological disorder which the authors have termed "low temperature breakdown".

SYMPTOMS

The chief symptom of this disorder is the development of diffuse, discoloured blotches in the flesh of the tuber (Plates I and II). In the initial stages these blotches are light reddish-brown in colour, and dark brown or almost black in advanced stages. The discoloration appears first at the stem end and may spread throughout the whole tuber. Small cavities, caused by the separation of the tissues, frequently appear in severely affected areas, but these are not confined to the central pith region as with hollow heart. In some cases the breakdown resembles late blight tuber rot, but the affected tissues are not dry and the discoloration does not originate at the surface and advance inwards as it does following late blight infection. Tissues affected by low temperature breakdown remain firm, not disintegrating as with bacterial soft rot, and there is no odour. The symptoms of low temperature breakdown are quite distinct from those produced by actual freezing of the tissues, as described by Jones *et al.* (4), Wright and Diehl (5), and Hurst (3), there being no distinct vascular necrosis, bluish-black discoloration, or leaking. The symptoms of low temperature breakdown are identical with those described by Hilborn and Bonde (2) and Folsom (1) for the type of low temperature injury that they called "internal mahogany browning".

In some varieties, notably the Katahdin, an external symptom of low temperature breakdown may be observed, a metallic, brownish-black colour appearing in irregular diffuse patches on the skin. The surface, however, is not depressed in these affected areas as it is on tubers infected with late blight. Surface discoloration is not an infallible criterion of low temperature breakdown, since it is not always present or it may be obscured by or confused with scuffing or other injuries to the skin.

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VARIETAL TESTS

In the course of these studies, six potato varieties were investigated for susceptibility to low temperature breakdown by storing them in refrigerated chambers held at 32°, 36°, and 39° F. throughout the winter months. At the end of the storage period, the tubers were cut longitudinally and examined for low temperature breakdown. The type of injury found was classified as slight, moderate, or severe, and then the following formula was applied to obtain an index, or rating, for each sample:

$$\frac{(\text{slight} \times 1)\ (\text{moderate} \times 2)\ (\text{severe} \times 3) \times 100}{\text{total number examined}} = \text{index}$$

This gave a range of indices from zero (no breakdown) to 300 (100 per cent severe breakdown).

In Table 1 the six varieties are listed in order of decreasing susceptibility to low temperature breakdown, according to the indices obtained in repeated tests. The varieties Katahdin, Chippewa and Irish Cobbler are considered to be very susceptible since they showed severe injury when stored at 32° F. and slight to moderate injury at 36° F. The variety Dooley (Rural New Yorker) is also considered to be susceptible although no injury was found after storage at 36° F. in these tests. The varieties Green Mountain and Warba are quite resistant to the disorder since only slight injury occurred at 32° F. In one test a trace of internal browning appeared in Warba tubers stored at 36° F., but it was not typical and it is doubtful if this was the result of the temperature experience.

Hilborne and Bonde (2) and Folsom (1) found that Katahdin and Chippewa tubers were severely affected by internal mahogany browning when stored at low temperatures, whereas Green Mountain tubers were not affected or only slightly so. Folsom (1) also found that predisposition to internal mahogany browning varied from one commercial or seedling variety to another, and that this predisposition is inherited.

EFFECT OF PACKING TUBERS IN MOIST SPHAGNUM MOSS

Tubers packed in moist sphagnum moss were compared with tubers not so protected, after storage at the same temperature, and found to be less affected by low temperature breakdown. For example, in Katahdin tubers stored at 32° F., the index of breakdown for those packed in moss was 82 whereas the index for the unprotected tubers was 221. Comparable results were obtained with tubers of the varieties Chippewa, Irish Cobbler, Dooley, Warba and Green Mountain. This reduction in the amount of breakdown is believed to be the result of the insulating effect of the moss. Internal temperatures of tubers, determined by inserting thermocouples, indicated an increase of from 0.5° to 1.0° F. in potatoes covered with damp moss. Hence the reduction of breakdown may be regarded as a temperature, rather than a moisture effect.

It was also noted that tubers packed in moist sphagnum showed more surface discoloration, while surface moulds developed to a less degree on them.

The protective effect of damp moss was consistently demonstrated in further tests that will be described later.

PLATE I. Three stages in development of low temperature breakdown in Katahdin tubers. *Left*, moderate; *centre*, severe; *right*, slight.

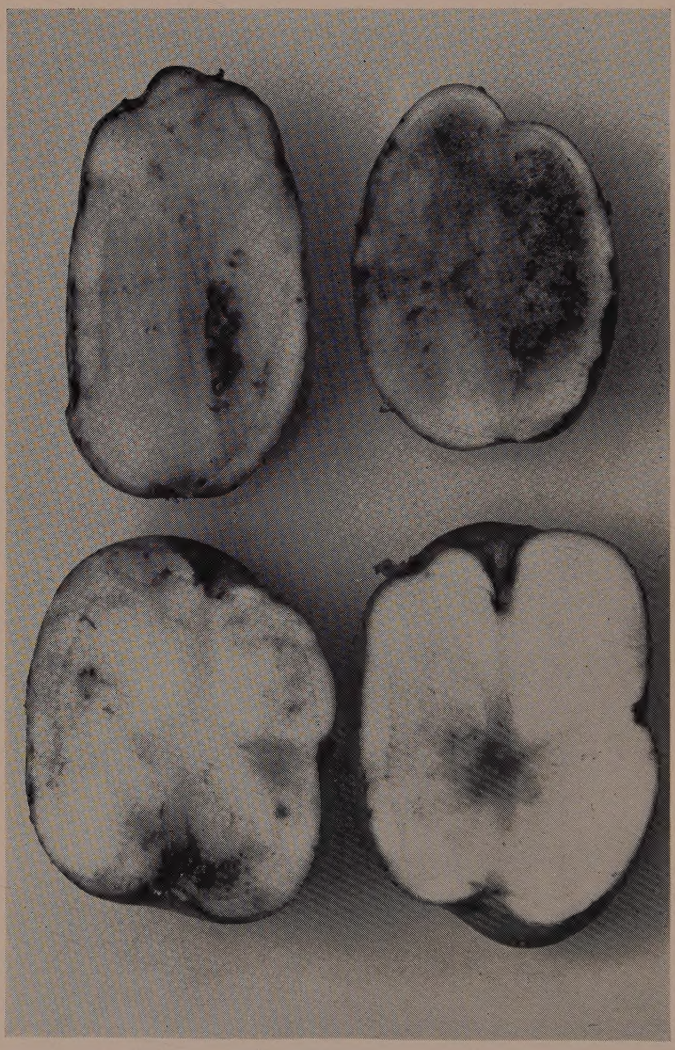

PLATE II. Low temperature breakdown in tubers of four potato varieties. *Upper left*, Dooley; *upper right*, Chippewa; *lower left*, Irish Cobbler; *lower right*, Warba.

TABLE 1.—LOW TEMPERATURE BREAKDOWN INDICES FOR SIX POTATO VARIETIES STORED AT 32°, 36° AND 39° F. FOR 7 MONTHS

Variety	Year	Low temperature breakdown index		
		32° F.	36°F.	39° F.
Katahdin	1941-42	300	0	0
	1942-43	245	41	0
	1943-44	221	0	0
	1944-45	140	20	0
	1945-46	132	0	0
	Average	208	13	0
Chippewa	1942-43	150	0	0
	1943-44	300	8	0
	Average	225	4	0
Irish Cobbler	1941-42	130	14	0
	1942-43	275	28	0
	1943-44	152	0	0
	Average	186	14	0
Dooley	1941-42	80	0	0
	1942-43	225	0	0
	1943-44	100	0	0
	Average	135	0	0
Warba	1941-42	9	0	0
	1942-43	50	0	0
	1943-44	16	12*	0
	Average	25	4*	0
Green Mountain	1941-42	16	0	0
	1942-43	33	0	0
	1943-44	0	0	0
	1945-46	5	0	0
	Average	14	0	0

* Doubtful.

LOSS IN WEIGHT BY TUBERS IN STORAGE

Calculations were made of loss in weight of tubers of six varieties under various storage treatments. These figures are recorded in Table 2.

The most striking fact found was the excessive loss in weight at 32° F. of those varieties susceptible to low temperature breakdown. For example, Irish Cobbler tubers lost 8 per cent of their weight at 32° F. whereas they lost less than 1 per cent at 36° F. and about 3 per cent at 39° F. On the other hand, tubers of the resistant varieties Green Mountain and Warba lost in the neighbourhood of 1 per cent of their weight at 32° F. As far as temperature is concerned, the general effect was that weight losses were least at lower temperatures except with varieties in which injury occurred.

The damp moss treatment was instrumental in conserving weight where the tubers were not affected by breakdown. In some exceptional cases, slight gains in the weight of the tubers were noted.

EFFECT OF CHANGING THE TEMPERATURE DURING STORAGE

Experiments were conducted to determine whether potato tubers react differently to low temperature at the beginning of their storage period than at the end of it. For this purpose, tubers of the susceptible variety Katahdin were put into storage, either dry or packed in moist sphagnum, and subjected to either an ascending or a descending temperature experience.

TABLE 2.—PERCENTAGE LOSS IN WEIGHT OF SIX POTATO VARIETIES KEPT IN STORAGE AT 32°, 36°, AND 39° F., DRY AND PACKED IN MOIST SPHAGNUM MOSS

Variety	Temperature	Dry treatment			Damp treatment		
		4 mo.	5 mo.	6 mo.	4 mo.	5 mo.	6 mo.
	°F.	%	%	%	%	%	%
Warba	32	1.9	2.2	2.8	0.3	0.6	1.4
	36	2.2	2.5	2.8	0.6	1.1	1.3
	39	2.8	3.4	3.9	*4.8	*4.4	*3.9
Dooley	32	4.4	5.5	7.0	1.4	2.5	3.5
	36	3.3	4.0	4.5	*1.3	*0.5	0.0
	39	4.3	5.0	5.1	1.6	2.5	3.6
Katahdin	32	2.7	4.6	7.1	0.9	3.6	5.4
	36	3.6	4.6	4.5	*0.5	0.0	0.0
	39	*2.7	*1.8	*0.9	0.0	0.9	0.9
Green Mountain	32	2.9	2.4	2.9	*0.3	0.0	0.8
	36	1.6	2.0	2.4	*0.4	0.0	0.4
	39	2.1	2.6	3.1	1.2	1.7	2.1
Irish Cobbler	32	2.2	4.7	7.2	2.5	5.6	7.9
	36	1.6	2.2	2.5	*0.6	0.0	0.3
	39	1.7	2.2	2.8	0.6	1.1	1.7
		2½ mo.	3½ mo.	4½ mo.	2½ mo.	3½ mo.	4½ mo.
Chippewa	32	1.0	1.5	2.0	*0.6	*0.5	0.0
	36	1.3	1.8	2.1	*0.1	*0.5	0.0
	39	1.2	2.0	2.5	0.0	0.7	1.0

* Percentage increase in weight.

In the first test, made in 1942-43, the ascending series were held 53 days at 32° F., 50 days at 36° F., and 53 days at 39° F.; the descending series were held 53 days at 39° F., 50 days at 36° F., and 53 days at 32° F. Control samples were held at 32° F. throughout the test period. When the tubers were examined, no low temperature breakdown was found in either the dry or the damp samples of the ascending or the descending series. The dry control had a low temperature breakdown index of 125 and the damp control an index of 50 at the end of the test period. It was concluded that an exposure of longer than 53 days would be required in order to induce symptoms of low temperature breakdown.

The experiment was repeated the following year, allowing longer periods of exposure to each temperature. The ascending series were exposed to 32° F. for 67 days, to 36° F. for 63 days, and to 39° F. for 73 days; the order of these temperatures was reversed for the descending series. The control series were held at 32° F. as before.

When the tubers were examined at the end of the storage period, the indices of low temperature breakdown for the various series were as follows:

Ascending	(32°→ 39°)	dry:	78
		damp:	21
Descending	(39°→ 32°)	dry:	10
		damp:	0
Control	(32°)	dry:	221
		damp:	82

Under the conditions of this experiment, the ascending temperature experience was more detrimental than the descending experience. In other words, exposure to the critical temperature (32° F.) for 67 days at the beginning of the storage period caused more damage through low temperature breakdown than exposure to this temperature for 73 days at the end of the storage period.

The protective effect of moist sphagnum moss was strikingly demonstrated throughout this experiment. Another observation made was that tubers of the ascending series had developed a sweeter flavour than those of the descending series. Also, exposure to low temperature at the end of the storage period (descending series) retarded bud development more than the same exposure at the beginning (ascending series).

PERIODIC EXAMINATION OF TUBERS IN STORAGE

In order to determine the point of onset of low temperature breakdown and to follow the course of its development, samples of tubers were examined periodically throughout the storage period.

Test in 1943-44

In the first of these tests, Katahdin tubers, both dry and packed in moist sphagnum moss, were put into storage on October 28, 1943, and held at 32° F. Examinations of 25 tubers from each lot were made on February 9, March 7, April 7, and May 17, 1944.

At the time of the first examination, breakdown was evidently just beginning in the damp sample, but had already reached an index of 88 in the dry sample (See Table 3). The course of development of the disorder in both lots from that point is indicated by increasing indices. The protective effect of the moist sphagnum was once again demonstrated. It may also be seen from Table 3 that the dry samples had a sweeter flavour than the corresponding samples packed in moss.

Test in 1944-45

In order to determine the point of onset of breakdown more accurately and to find some means to determine or explain its occurrence, the experiment was repeated the following year, when more frequent examinations were made over the entire storage period. Katahdin tubers were put into the storage chambers on November 1, 1944, and held at 32°, 36°, and 39° F. until May 21, 1945. Half of the tubers kept at 32° F. and 36° F. were

TABLE 3.—RESULTS OF PERIODIC EXAMINATION OF KATAHDIN TUBERS STORED AT 32° F. DRY AND PACKED IN MOIST SPHAGNUM MOSS (1943-44)

Storage period (days)	Dry series		Damp series	
	Low temperature breakdown index	Flavour	Low temperature breakdown index	Flavour
105	88	M.S.[2]	4	S.S.[1]
131	136	V.S.[3]	32	S.S.
159	212	V.S.	60	S.S. to M.S.
202	221	V.S.	82	M.S.

[1]S.S. = slightly sweet. [2] M.S. = moderately sweet. [3]V.S. = very sweet.

packed in moist sphagnum, while the remainder, and those at 39° F., were left unprotected. At weekly intervals, samples of 10 tubers from each lot were removed, examined externally and internally, and tasted for sweetness. On alternate weeks, refractometer tests for soluble solids in the expressed juice were made and cooking tests were conducted.

Since only 10 tubers were used for each examination, there was considerable variation in the indices obtained for surface discoloration. However, when the indices from 24 examinations were averaged, as shown in Table 4, the relationships between temperature and moisture and surface discoloration became apparent. Surface discoloration appeared after 7 weeks on the tubers stored at 32° F., about the same time as internal breakdown, but did not subsequently progress to the same extent as the breakdown. Surface discoloration appeared to a less degree at the higher temperatures (after 13 weeks at 36° F., and after 15 weeks at 39° F.), but was not accompanied by breakdown. Thus it would seem that surface discoloration, although caused by exposure to low temperatures, is independent of the breakdown. It was also noted that more surface discoloration developed in samples packed in moist sphagnum moss than in the dry samples.

The growth of saprophytic moulds on surface wounds was greatest on tubers stored at 39° F. and least on those stored at 32° F. (Table 4). It seems probable that temperature, rather than the condition of the tubers, was the factor governing the development of these fungi. Moulds developed less extensively on tubers packed in moist sphagnum than on those stored dry, suggesting that the moss had a retarding effect despite the more favourable conditions of humidity which it provided.

The first internal symptoms of low temperature breakdown were found at the 51-day examination in the tubers stored dry at 32° F. (Figure 1), and at 85 days in the tubers packed in moss at this temperature. The indices of breakdown for both lots appeared to increase rapidly after onset to a near maximal point, then only slightly thereafter, but the samples were too small to give a smooth progress curve. A trace of breakdown appeared, at 36° F., after 177 days in the dry samples and after 184 days in the damp samples. No breakdown developed in the tubers stored at 39° F. during the test period (201 days).

TABLE 4.—INDICES OF SURFACE DISCOLORATION AND SURFACE MOULDS ON KATAHDIN TUBERS STORED UNDER VARIOUS CONDITIONS, BASED ON 24 PERIODIC EXAMINATIONS OF 10-TUBER SAMPLES

Storage temperature, ° F.	Storage moisture	Surface discoloration (average index)	Surface moulds (average index)
32	Dry	28	57
32	Damp	40	50
36	Dry	18	64
36	Damp	22	40
39	Dry	3	82

The trend of the refractometer readings for soluble solids is shown in Figure 1. It is assumed that the increments mainly represent increase in sugar accumulation or production from starch under low temperature conditions. The trend was distinct for each temperature, and, throughout, the dry samples gave slightly higher readings than the corresponding damp ones. At the lowest temperature (the one that induced the earliest onset and the greatest amount of breakdown) the greatest increase in sugar was found. As indicated on the graph (Figure 1), breakdown commenced at a refractometer reading above 9 per cent. At this point the raw tubers became definitely sweet to the taste.

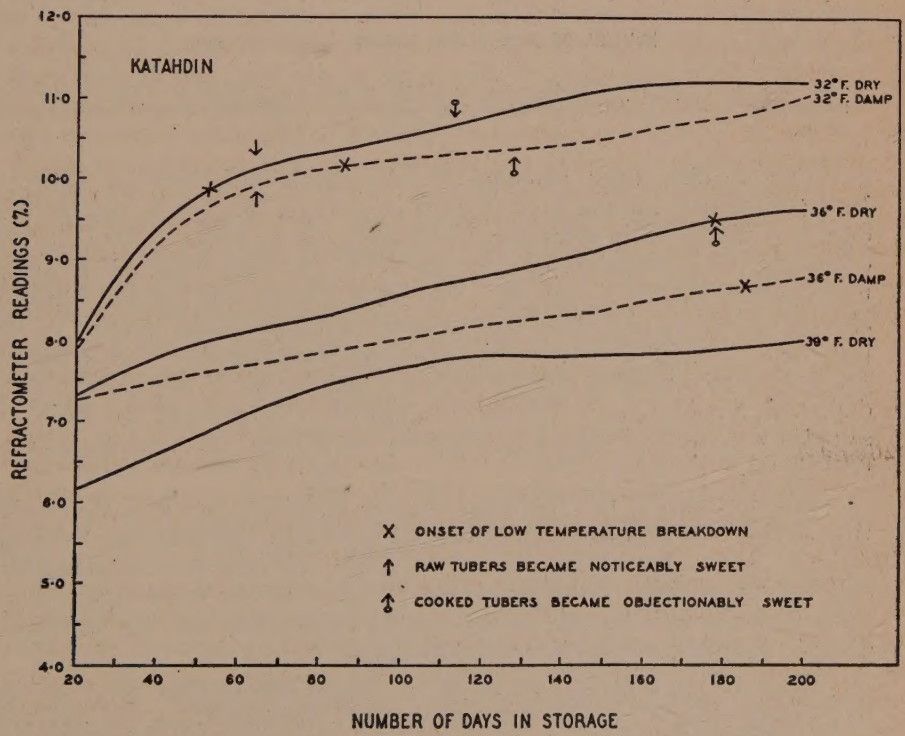

FIGURE 1. The respective trends in concentration of soluble solids in the expressed juice of Katahdin tubers held at 32°, 36°, and 39° F., either dry or packed in moist sphagnum moss for 201 days (1944-45). The point of onset of low temperature breakdown and changes in flavour are also indicated.

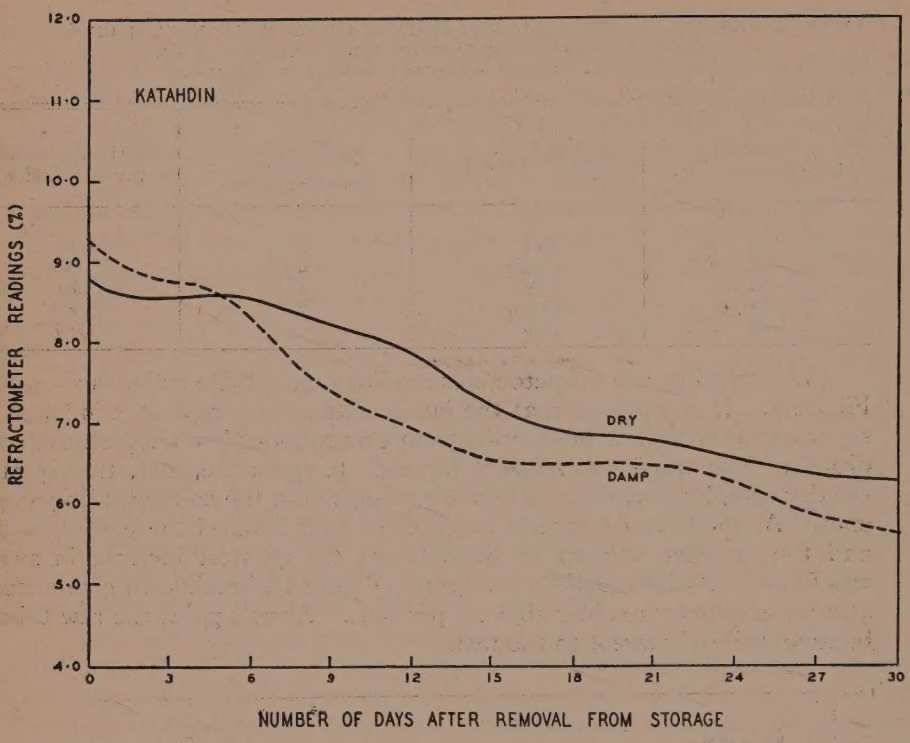

FIGURE 2. Trends in concentration of soluble solids in expressed juice of Katahdin tubers held at 65° F. and 95 per cent R.H. for 30 days following 91 days in storage at 32° F.

The cooking tests revealed no detectable difference in sweetness between samples for the first 64 days in storage, in spite of the fact that extreme differences in refractometer readings were found. After 78 days of storage, all material except that at 39° F. was sweet, the 32° F. sample more so than the 36° F. sample. The 32° F. dry sample became objectionably sweet after 115 days of storage, with a refractometer reading of 10.8 per cent. The 32° F. damp sample became objectionable when cooked at 125 days with a reading of 10.2 per cent, and the 36° F. dry sample at 175 days with a reading of 9.5 per cent. The 36° F. damp and the 39° F. dry samples were still of satisfactory quality at the end of the storage period (201 days).

One interesting fact revealed by the cooking tests was that tubers that showed the slightest sign of breakdown in the raw state turned black, at least in the affected portions, either during cooking or when allowed to stand after cooking. Thus prolonged exposure to low temperature during storage might well be one of the factors responsible for the undesirable discoloration so common in cooked potatoes.

After 78 days in storage at 32° F., some tubers were kept at room temperature for 3 days and then cooked. It was found that the sweetness still persisted. To ascertain whether or not the sugar concentration could still be decreased, tubers that had been stored at 32° F. for 91 days were kept at room temperature for a month during which refractometer read-

ings were taken regularly. The results, as illustrated in Figure 2, indicated that the sugar concentration can still be decreased, even at this stage of storage, either through respiration or reconversion to starch, or both. No cooking test was conducted on this material, but the raw tubers were found to be only slightly sweet on the 15th day. It is a curious fact that while the sample stored in damp moss had a higher concentration of sugar than the dry sample when taken from storage, the decrease in sugar in the damp sample took place at a more rapid rate, resulting in a lower concentration that that of the dry sample from the 7th to the 30th day.

The outstanding conclusion that can be drawn from the data obtained from the periodic examinations in the 1944-45 tests is that, at least in the Katahdin variety, there is a definite correlation between the incidence of low temperature breakdown and the sugar content of the tubers as indicated by the refractometer and flavour tests.

Test in 1945-46

The periodical examinations were repeated in 1945-46 with the variety Katahdin in order to further ascertain the relationship between low temperature breakdown and sugar content of the tubers. The resistant variety Green Mountain was also used for comparison. Tubers of both varieties were put into storage chambers on November 15, 1945 (after 6 weeks in common storage), and held at 32°, 36°, and 39° F. until May 11, 1946. The damp moss treatment was not used in this test. Examinations were made at 2-week instead of weekly intervals, but larger samples (60 tubers) were taken each time with a view to obtaining more consistent data on the development of breakdown. Observations made in the course of this test are summarized in Tables 5, 6, and 7.

Surface discoloration developed earliest and to the greatest extent in Katahdin tubers stored at 32° F., and latest and to the least extent in those stored at 39° F. No surface discoloration developed in Green Mountain tubers stored at any of the three temperatures. In both varieties the growth of moulds on surface wounds varied directly with the temperature, again indicating that temperature had a more direct effect than the condition of the tubers on the development of these saprophytic fungi. Moulds developed more extensively on Katahdin tubers than on Green Mountain tubers stored at the same temperature.

The flavour tests indicated that tubers of both varieties stored at 32° F. rapidly developed sweetness, those at 36° F. more slowly and to a less degree, while those at 39° F. did not become sweet at all. In each treatment the Green Mountain tubers tasted sweeter than the Katahdin tubers stored under the same conditions.

The increase in sweetness, indicating accumulation of sugar presumably derived from starch, was accompanied by an increase in soluble solids, as indicated by the refractometer readings taken on samples of juice expressed from the tubers. The trends of the refractometer readings throughout the storage period are shown in Figure 3. The trends were distinct for each temperature, and the reading for Green Mountain tubers was higher, in practically every case, than the corresponding reading for Katahdin tubers.

TABLE 5.—RESULTS OF PERIODIC EXAMINATION OF KATAHDIN AND GREEN MOUNTAIN TUBERS STORED AT 32° F. (1945-46)

Storage period (days)	Katahdin					Green Mountain				
	Surface discoloration index	Surface moulds index	Flavour	Refractometer reading (%)	Low temperature breakdown index	Surface discoloration index	Surface moulds index	Flavour	Refractometer reading (%)	Low temperature breakdown index
0	—	—	—	4.9	0	—	—	—	4.8	0
16	—	—	—	6.0	0	—	—	—	6.8	0
31	—	—	—	8.2	0	—	—	—	8.4	0
45	—	—	—	9.4	0	—	—	—	9.1	0
59	0	52	S.[1]	9.0	2	0	35	V.S.[2]	10.5	0
73	0	28	S.	9.0	0	0	27	V.S.	10.2	0
87	0	27	S.	9.0	23	0	15	V.S.	10.4	0
101	27	73	S.	9.8	35	0	10	V.S.	10.6	0
115	50	88	S.	9.2	55	0	20	V.S.	10.0	0
129	75	78	V.S.[2]	10.2	126	0	13	V.S.	9.9	2
143	113	103	V.S.	9.7	93	0	10	V.S.	9.8	5
178	152	111	V.S.	10.3	132	0	27	V.S.	9.9	0

[1] S. = Sweet. [2] V.S. = Very sweet.

TABLE 6.—RESULTS OF PERIODIC EXAMINATION OF KATAHDIN AND GREEN MOUNTAIN TUBERS STORED AT 36° F. (1945-46)

Storage period (days)	Katahdin					Green Mountain				
	Surface discoloration index	Surface moulds index	Flavour	Refractometer reading (%)	Low temperature breakdown index	Surface discoloration index	Surface moulds index	Flavour	Refractometer reading (%)	Low temperature breakdown index
0	—	—	—	4.9	0	—	—	—	4.8	0
16	—	—	—	6.2	0	—	—	—	7.8	0
31	—	—	—	6.7	0	—	—	—	7.9	0
45	—	—	—	6.9	0	—	—	—	7.6	0
59	0	47	N.S.[1]	6.8	0	0	25	S.S.	7.7	0
73	0	52	S.S.[2]	6.6	0	0	45	S.S.	7.2	0
87	0	17	S.S.	6.6	0	0	33	S.S.	7.4	0
101	0	32	S.S.	6.8	0	0	48	S.S.	7.1	0
115	27	73	S.S.	7.0	0	0	13	S.S.	7.8	0
129	55	75	S.S.	7.0	0	0	5	S.S.	7.4	2 (?)
143	78	78	S.S.	6.2	0	0	23	N.S.[1]	6.4	0
178	39	48	S.S.	7.6	0	0	4	S.[3]	7.1	0

[1] N.S. = Not sweet. [2] S.S. = Slightly sweet. [3] S = Sweet.

TABLE 7.—RESULTS OF PERIODIC EXAMINATION OF KATAHDIN AND GREEN MOUNTAIN TUBERS STORED AT 39° F. (1945-46)

Storage period (days)	Katahdin					Green Mountain				
	Surface discoloration index	Surface moulds index	Flavour	Refractometer reading (%)	Low temperature breakdown index	Surface discoloration index	Surface moulds index	Flavour	Refractometer reading (%)	Low temperature breakdown index
0	—	—	—	4.9	0	—	—	—	4.8	0
16	—	—	—	5.8	0	—	—	—	6.2	0
31	—	—	—	6.2	0	—	—	—	6.8	0
45	—	—	—	6.0	0	—	—	—	7.0	0
59	0	142	N.S.[1]	6.4	0	0	51	N.S.[1]	7.0	0
73	0	117	N.S.	6.2	0	0	30	N.S.	6.6	0
87	0	83	N.S.	6.2	0	0	30	N.S.	6.8	0
101	0	105	N.S.	6.2	0	0	20	N.S.	6.6	0
115	15	55	N.S.	6.7	0	0	37	N.S.	7.0	0
129	33	55	N.S.	6.4	0	0	65	N.S.	6.8	0
143	53	47	N.S.	6.3	0	0	27	N.S.	6.8	0
178	22	52	N.S.	6.7	0	0	53	N.S.	6.9	0

[1] N.S. = Not sweet.

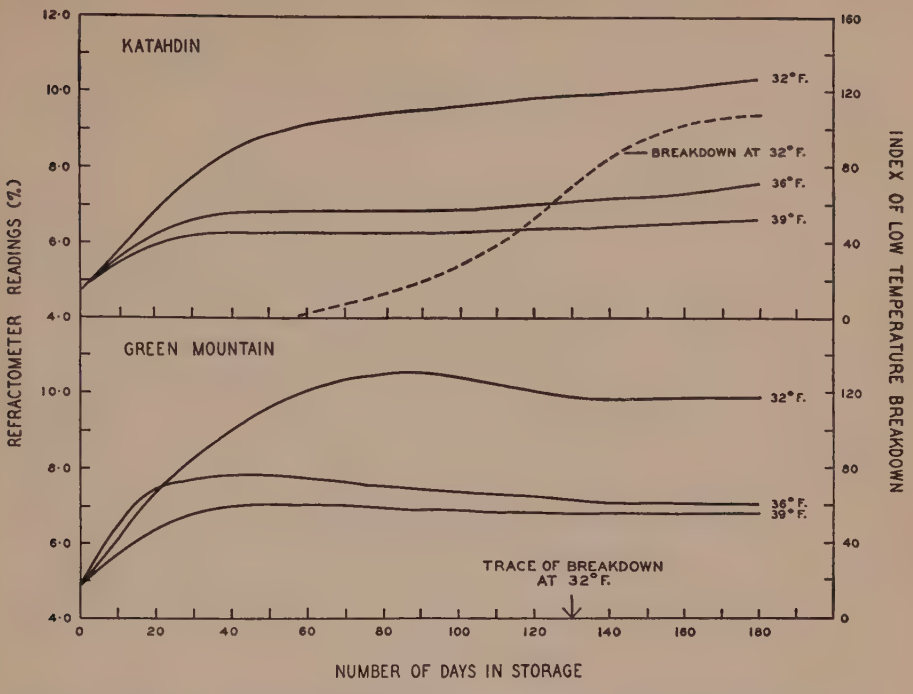

FIGURE 3. Trends of soluble solids concentration and low temperature breakdown in Katahdin and Green Mountain tubers during 178 days in storage at 32°, 36°, and 39° F. (1945-46).

Severe low temperature breakdown developed only in Katahdin tubers stored at 32° F. (Tables 5, 6, and 7). The first evidence of breakdown in this variety appeared at 59 days, and the trend of its development is shown in Figure 3. The refractometer reading for the Katahdin tubers at 32° F. was 9.0 per cent when breakdown commenced, while samples stored at 36° and 39° F. did not reach this level at any time during the test. This confirms the observation made in the previous test that low temperature breakdown and sugar concentration in Katahdin tubers are correlated. However, a high concentration of sugars is not always accompanied by breakdown. Tubers of the resistant variety Green Mountain gave consistently higher refractometer readings throughout than did the Katahdin tubers, yet only a trace of breakdown developed in these, and that after 129 days in storage at 32° F.

OTHER INTERNAL DISORDERS

In the course of these experiments, various other internal disorders were encountered, but none could be attributed to the low temperature experience of the tubers. The disorders included stem-end browning, vascular necrosis, bacterial soft rot, Fusarium dry rot, and hollow heart. Tubers severely affected by low temperature breakdown were ultimately invaded by typical storage rot organisms. Careful notes were taken on the incidence of hollow heart, but it was found to be independent of the storage experience.

EFFECT OF STORAGE ON SUBSEQUENT GROWTH

With seed potatoes, it is vitally important to know what the effect of the storage experience will be on subsequent germination, growth, and yield. For this reason, these storage tests were followed by field tests in order to obtain this information.

Bud Development During Storage

At the time of the final examination of each lot of tubers in storage, particular attention was paid to the condition of the buds. The observations on bud development at the end of the 1942-43 storage test are shown in Table 8; essentially the same conditions were observed in other years. In all varieties, the eyes of tubers stored at 32° F. were still dormant at the end of the storage period. The buds of some of the tubers of the varieties Katahdin and Irish Cobbler (which are very susceptible to low temperature breakdown) were dead when examined. Tubers of most of the varieties remained dormant at 36° F., but none of the buds was killed. Buds developed slightly on tubers of all varieties stored at 39° F. In general, the buds on tubers packed in moist sphagnum moss were more advanced than those on corresponding tubers stored dry. The increase in moisture provided by the moss as well as the slightly higher temperature noted previously would favour bud development.

Germination in the Field

The percentage of germination in planted tubers of those varieties susceptible to low temperature breakdown was drastically reduced by storage at 32° F., as indicated in Table 8, whereas it was essentially normal for the resistant varieties Warba and Green Mountain. Tubers of all varieties stored at 36° F. germinated equally as well as those stored at 39° F.

Growth of Plants

The storage experience of the tubers had a marked effect on the growth of the plants. After storage under conditions conducive to low temperature breakdown, tubers of susceptible varieties produced an irregular stand of plants, many of which were stunted or spindly. The irregularity in the size of these plants was evidently the result of retarded germination and of early decay of the seed pieces. Tubers of resistant varieties produced normal plants regardless of the storage conditions.

Yield of Tubers

Storage at low temperature reduced the yield in those varieties susceptible to low temperature breakdown in two ways: fewer plants developed from the sets planted; the plants that did grow were subnormal. In Table 8, the yield data for the 1942-43 planting are expressed in two ways: the yields given on the basis of pounds per hill indicate the differences in the crop due to the differences in vigour of the plants; the yields given on the basis of pounds per 100 sets planted indicate the combined effect of germination and vigour.

While these plantings were not replicated each year to compensate for soil differences, the results obtained in four different growing seasons

TABLE 8.—GERMINATION AND YIELD DATA ON TUBERS OF SIX VARIETIES PLANTED AFTER 7 MONTHS' STORAGE AT 32°, 36°, AND 39° F. DRY AND PACKED IN MOIST SPHAGNUM MOSS (1942-43)

Variety	Storage temperature, ° F.	Storage moisture	Condition of eyes	Germination (%)	Vigour of plants	Yield	
						Lb. per plant	Lb. per 100 sets
Katahdin	32	Dry	Dormant or dead	50.2	Poor	0.7	20
		Damp	Dormant or dead	41.7	Fair	1.0	40
	36	Dry	Dormant	96.1	Fair	0.6	43
		Damp	Dormant	94.0	Normal	0.8	63
	39	Dry	Very slightly budded	92.5	Normal	0.7	54
		Damp	Very slightly budded	98.7	Normal	0.8	73
Chippewa	32	Dry	Dormant	92.8	Poor	0.7	49
		Damp	Dormant	99.0	Poor	0.8	67
	36	Dry	Dormant	98.0	Normal	0.8	65
		Damp	Dormant	100.0	Normal	1.1	89
	39	Dry	Slightly budded	98.0	Normal	0.9	64
		Damp	Slightly budded	100.0	Normal	1.2	95
Irish Cobbler	32	Dry	Dormant or dead	37.9	Poor	0.9	42
		Damp	Dormant or dead	47.2	Poor	1.2	45
	36	Dry	Dormant	95.3	Fair	0.8	68
		Damp	Dormant	96.0	Fair	0.9	82
	39	Dry	Slightly budded	100.0	Normal	0.9	75
		Damp	Slightly budded	99.3	Normal	1.0	85
Dooley	32	Dry	Dormant	75.0	Poor	0.7	49
		Damp	Dormant	86.0	Poor	1.0	78
	36	Dry	Dormant	97.0	Fair	0.8	73
		Damp	Slightly budded	100.0	Fair	0.8	66
	39	Dry	Slightly budded	93.0	Normal	0.9	82
		Damp	Slightly budded	100.0	Normal	1.1	110
Warba	32	Dry	Dormant	96.6	Fair	1.1	106
		Damp	Dormant	99.3	Fair	1.1	95
	36	Dry	Dormant	99.3	Normal	1.2	120
		Damp	Dormant	100.0	Normal	1.1	93
	39	Dry	Slightly budded	99.3	Normal	1.3	115
		Damp	Slightly budded	100.0	Normal	1.0	90
Green Mountain	32	Dry	Dormant	98.0	Fair	1.3	116
		Damp	Dormant	100.0	Normal	1.2	106
	36	Dry	Dormant	100.0	Normal	1.1	96
		Damp	Very slightly budded	97.0	Normal	1.3	98
	39	Dry	Very slightly budded	100.0	Normal	1.2	112
		Damp	Slightly budded	98.0	Normal	1.3	123

were quite consistent and permit certain conclusions to be drawn. Tubers of varieties susceptible to low temperature breakdown that had been stored at 32° F. produced the lowest yield per plant as well as total yield, while those stored at 39° F. produced the highest yields. The yields following storage at 36° F. were close to those following storage at 39° F. The resistant varieties Warba and Green Mountain gave comparable yields following storage at all three temperatures used. In general, tubers that had been packed in moist sphagnum moss gave higher yields at all temperatures than those that had not been protected. This was particularly true of the susceptible varieties.

In two of the field tests, half of the tubers in each lot was kept at room temperature for one week before planting while the other half was left in the storage chambers until the day they were planted. Slightly higher yields were obtained in most cases from the tubers that were planted immediately on removal from storage. The difference was possibly due to deterioration of the tubers (particularly those injured by low temperature) by secondary rots developing at the higher temperature. In actual practice, this increase in yield might not be so important as the earlier maturity derived from holding seed potatoes at warmer temperatures before planting to promote sprout development.

Hilborne and Bonde (2) observed that reduced stands and yields resulted when Katahdin and Chippewa tubers affected by low temperature injury were planted in the fields.

DISCUSSION

These investigations have revealed that tubers of certain potato varieties may be seriously damaged by prolonged exposure to temperatures which are not low enough actually to freeze the tissues. Such exposure can be injurious to both table stock and seed potatoes: Table stock may be rendered objectionable in both appearance and flavour; seed potatoes may germinate poorly and produce subnormal plants and a reduced yield.

The ideal storage temperature for most varieties appears to be 36° F. At this temperature little if any breakdown occurs, yet there is a minimum loss in weight. Sprouts will not develop too rapidly at 36° F., yet they are not killed or retarded too much for seed purposes. Tubers can be stored at 36° F. for long periods without becoming objectionably sweet, so this temperature should be satisfactory even for table stock potatoes. Every effort should be made to store seed potatoes at temperatures as near 36° F. as possible, and definitely not lower than that for any length of time.

The temperature in some common storage buildings, both commercial and domestic, is allowed to fall below 36° F. and to remain there for periods sufficiently long for low temperature breakdown to develop in susceptible varieties of potatoes. As shown in these studies, an exposure to 32° F. of two months' duration at any time in the storage life of the tubers is sufficient to induce breakdown in susceptible varieties, such as Katahdin, Chippewa, Irish Cobbler, and Dooley. The resistant varieties, such as Green Mountain and Warba, will withstand this temperature much longer without injury.

No differences were found between tubers of the various varieties that would account for the differences in susceptibility to low temperature breakdown. This disorder was found to occur in Katahdin tubers when the cells had a high sugar content, as indicated by taste and refractometer tests, but Green Mountain tubers developed even higher sugar concentrations without suffering injury. A comparison of respiration rates in resistant and susceptible varieties at different temperatures might throw some light on the problem. It is also possible that enzyme action might be involved in the development of the disorder.

The fact that tubers affected by low temperature breakdown turned dark during or after cooking, suggests that prolonged exposure to low temperature may be one of the causes of blackening in cooked potatoes.

The protective effect of moist sphagnum moss is interesting from the experimental standpoint, but this treatment is not likely to prove a practical means of preventing low temperature breakdown in storage. However, the results obtained do indicate the advantage of maintaining a high relative humidity throughout the storage life of potatoes.

SUMMARY

1. A physiological disorder termed "low temperature breakdown" occurs when certain potato varieties are stored at temperatures just above the freezing-point of the tissues. The symptoms of this disorder are: (*a*) discoloured blotches in the flesh of the tuber varying from light reddish-brown to dark brown, and (*b*) a metallic, brownish-black colour appearing in diffuse patches on the skin.

2. The following varieties were tested and are listed in order of decreasing susceptibility to low temperature breakdown: Katahdin, Chippewa, Irish Cobbler, Dooley, Green Mountain and Warba. The last two varieties are highly resistant to the disorder.

3. Tubers packed in moist sphagnum moss were injured less when stored at low temperature than tubers not so protected. Surface moulds developed to a less degree on tubers packed in sphagnum.

4. Tubers of susceptible varieties lost weight excessively at 32° F. Loss in weight in resistant varieties varied directly with the temperature.

5. Exposure to 32° F. for two months at the beginning of the storage period was more deleterious to Katahdin tubers than similar exposure at the end of the storage period.

6. Periodic examinations of Katahdin and Green Mountain tubers stored at 32°, 36°, and 39° F. revealed that:

(*a*) Low temperature breakdown appeared in Katahdin tubers after 2 months at 32° F. and after 6 months at 36° F. A slight amount of breakdown appeared in Green Mountain tubers after $4\frac{1}{2}$ months at 32° F.

(*b*) Low temperature breakdown in Katahdin tubers was associated with high sugar concentrations, as indicated by refractometer tests. Green Mountain tubers showed even higher sugar concentrations, however, without showing symptoms of breakdown.

(*c*) Surface discoloration was most severe on Katahdin tubers stored at 32° F. and did not develop on Green Mountain tubers.

(*d*) The growth of moulds on surface wounds varied directly with the storage temperature.

(*e*) Tubers stored at 32° F. rapidly developed a sweet flavour; tubers at 36° F. were less sweet, and those at 39° F. did not become sweet. Green Mountain tubers tasted sweeter than Katahdin tubers stored under the same conditions.

(*f*) Tubers affected by low temperature breakdown turned black when cooked or allowed to stand after cooking.

7. Low temperature retarded or inhibited bud development in tubers of varieties susceptible to breakdown. Such tubers germinated poorly when planted, and produced subnormal plants with a correspondingly reduced yield.

8. A storage temperature of 36° F. is considered the best from all standpoints for the storage of potatoes, particularly if they are to be used for seed.

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EXPERIMENTS WITH RADIOPHOSPHORUS ON THE UPTAKE OF PHOSPHORUS BY WHEAT[1]

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The introduction of the tracer technique in the study of soil chemistry and plant nutrition has provided a new and valuable method for attacking these difficult fields of research. In particular, the tracer technique using radiophosphorus has many useful applications in studying the problems associated with chemical reactions of phosphorus in soil and fertilizer, and with the uptake of phosphorus by the growing plant.

The use of phosphatic fertilizers on crops in Saskatchewan has become common practice in recent years. Results of some experimental work have been reported (4) and further experiments have led to the conclusion that very general use of phosphate fertilizer on wheat and other crops is a requirement in obtaining the highest yields on most Dark Brown and Black soils (5) of the region. There are some 24 million acres of such soils under cultivation, a little over a third of the acreage being sown to summerfallow wheat each year. Present recommendations are to use phosphate fertilizer on summerfallow crops only, since other crop land generally gives little or no response. Considering that most of the 8 or 10 million acres of summerfallow may eventually be fertilized, and that the average increase in yield of wheat obtained is about 5 bushels per acre, the growing interest in phosphatic fertilizers may be readily understood.

The presently recommended fertilizer treatment is 11-48-0 at 30 to 60 pounds per acre. In spite of the fact that a nitrogen-carrying fertilizer is used, the element nitrogen seems to have no direct effect in producing the increased yield (4), and the advantage of 11-48-0 over other phosphates seems to be due to the greater efficiency of ammonium phosphate as a carrier of phosphorus when applied to the neutral or alkaline soils common to this area.

BRIEF REVIEW OF RESULTS OF RADIOPHOSPHORUS WORK AT THE UNIVERSITY OF SASKATCHEWAN

Results of experiments using radiophosphorus have been reported by Spinks and Barber (7, 8), Barber, Mitchell and Spinks (2), and by Spinks, Dion, Reade and Dehm (9). A review of literature is given in these publications and also the techniques developed in using radiophosphorus as a tracer element in studying the reactions of applied phosphorus in soil and plant.

Some of the more important conclusions reported in the above papers are as follows:

1. That the wheat plant takes up phosphorus from soil and fertilizer until maturity, but that the main absorption of the fertilizer phosphorus takes place well before the plant matures.

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[1] Contribution from Department of Soils, University of Saskatchewan, Saskatoon. Presented at meeting of Soils Subject Division, Agricultural Institute of Canada, Guelph, Ont., June, 1948.

[2] Associate Professor of Soils.

[3] Professor of Chemistry.

[4] Professor of Soils.

2. Where small applications of fertilizer were used on an Elstow silt loam soil (25 lb. per acre 11-48-0 in these experiments) about 22 per cent recovery by the plant was obtained. The per cent recovery tends to decrease as the amount of application is increased, although the actual amount of phosphorus taken up from the fertilizer may increase.

3. In earlier stages of growth, the larger amount of phosphorus taken up by the plant is obtained from the fertilizer; thereafter the soil supplies the greater portion.

4. In some cases, the fertilized wheat may use greater amounts of soil phosphorus than the unfertilized crop where light applications (25 lb. 11-48-0 per acre) are the practice. However, for heavier applications the reverse appears to hold true (9).

Barber (1) also studied the effect of time of application on phosphorus uptake, and on growth. His data were obtained in the greenhouse and indicate that applying the fertilizer at the two- and four-week stage increased the uptake of fertilizer phosphorus, but that there was a tendency for less increase in growth as compared to application with the seed.

These results are summarized in Table 1. They indicate that the practice of applying the fertilizer at seeding gives better yield results than would applications at any later stage of growth. This is fortunate since the most practicable time to apply fertilizer is when the grain is being seeded. According to Gericke (3) the critical time in phosphorus nutrition for the wheat plant appears to be during the first few weeks after emergence. It is possible that the importance of early supplies of phosphorus is connected with the early development of flower primordia in the young seedling. The maximum number of flowers in the spike is apparently determined at about the 4- to 6-week stage in plant development, and to have an effect on length of head and number of kernels, the necessary phosphorus must be present quite early in the plant's development.

The basic worth of the tracer method in this type of experimental work lies in the fact that the use of radiophosphorus permits the accurate identification of applied phosphorus in soils and plants. For instance, the amount of phosphorus recovered by the plant from a particular fertilizer has heretofore been estimated by comparing total phosphorus uptake by fertilized and unfertilized plants. This method does not exclude the

TABLE 1.—EFFECT OF TIME OF APPLICATION ON GROWTH AND UPTAKE OF PHOSPHORUS BY WHEAT PLANTS

(Plants harvested at 8 weeks)

Time of application	Weight of plants at 8 weeks (gm.)	Total P. in plant material (mg.)	P. in plant obtained from fertilizer (mg.)*
At seeding	3.17	9.51	2.86
Two weeks	2.67	7.93	3.03
Four weeks	2.79	8.19	3.48
Half at planting and half at four weeks	2.82	7.74	3.33
No fertilizer	2.57	5.50	—

* Difference of 0.54 mg. required for significance at 5 per cent point.

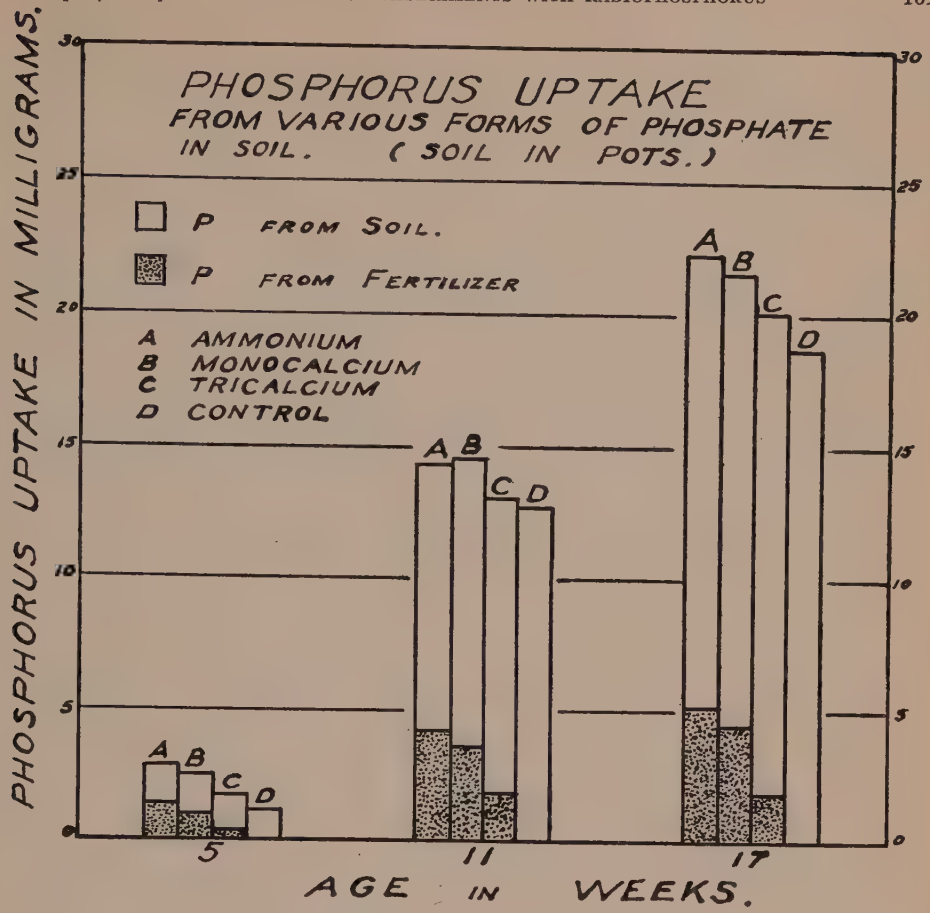

FIGURE 1. Showing uptake of phosphorus by wheat plants from several phosphate fertilizers at various stages of growth

possibility that the fertilized crop might use a different amount of soil phosphorus simply as a result of fertilization. Indeed, it appears (8) that for light applications more soil phosphorus may be taken up, but for very heavy applications, there may be less. The possibility that there may be an additional demand on the available phosphorus in some soils as a result of fertilizing with low rates of phosphate is a matter of considerable interest. In Saskatchewan and in the prairie provinces generally, light applications of from 20 to 30 pounds of 11-48-0 ammonium phosphate are usual, although larger applications are recommended.

In general, the fertilizer is applied to the summerfallow crop since stubble crops respond only in certain districts where moisture conditions are especially favourable. The average 20 to 30 pound application which the farmers are at present using will hardly balance the phosphorus requirements of the crops in the common three-year rotation of summerfallow, wheat and wheat or other grain. This, and the possibility that there may be a greater drain on the native phosphorus of the soil than had been suspected, points to a need for higher rates than are at present in use. Field experiments have shown that higher applications, on the average, give profitable increases up to 50-60 lb. per acre (6).

As previously mentioned, an ammonium phosphate appears to have a definite superiority over triple superphosphate or single superphosphates on the usually neutral to alkaline and frequently calcareous soil of this area (5). An experiment carried on in the greenhouse to test the relative availability of several forms of phosphate gives further information on this question.

Wheat was grown to the 5-week stage, to the early shot-blade (11 weeks) and to the dough stage (17 weeks) in pots fertilized with mono-ammonium, mono-calcium and tri-calcium phosphate, at rates equivalent to 25 lb. per acre of 11-48-0. The phosphates were prepared in the laboratory, and carried radiophosphorus as a tracer. The preparation of pure orthophosphates of calcium is a matter of some difficulty, and there was no assurance that the salts prepared were of a high degree of purity. However, on the basis of their analyses, it was felt that their performance in the availability trials would at least be a reflection of the quite different proportions of calcium combined with phosphorus in these two compounds,

TABLE 2.—COMPARATIVE UPTAKE OF PHOSPHORUS BY WHEAT FROM AMMONIUM PHOSPHATE, MONO-CALCIUM PHOSPHATE AND TRI-CALCIUM PHOSPHATE APPLIED TO ELSTOW SILT LOAM

Average results at 5-week stage

	$NH_4H_2PO_4$	$Ca(H_2PO_4)_2$	$Ca_3(PO_4)_2$	Unfert.	Least sig. difference at 5% point
Fert. P. taken up by plants (mg.)	1.4	1.0	0.3	—	0.1
Total P. in plants (mg.)	2.8	2.5	1.7	1.1	1.0
Soil P. in plants (mg.)	1.4	1.5	1.4	1.1	—
Weight plant material (gm.)	0.65	0.59	0.49	0.39	0.085

Averages at early shot-blade stage—11 weeks

Fert. P. taken up by plants (mg.)	4.2	3.6	1.9	—	0.3
Total P. in plants (mg.)	14.4	14.5	13.1	12.8	1.2
Soil P. in plants (mg.)	10.2	10.9	11.2	12.8	1.4
Weight plant material (gm.)	4.36	4.34	3.93	3.82	0.26

Averages at dough stage—17 weeks

Fert. P. taken up by plants (mg.)	5.2	4.5	1.9	—	0.4
Per cent recovery of Fert. P.	29.0	25.0	10.3	—	2.2
Total P. in plants (mg.)	22.3	21.6	20.2	18.8	2.0
Soil P. in plants (mg.)	17.1	17.1	18.3	18.8	1.8
Weight plant material (gm.)	9.01	8.91	8.71	7.77	0.32

and would give some information on the question of the availability of calcium phosphates in comparison to ammonium phosphates in a base-saturated soil.

The soil was Elstow silt loam, pH 7.1, (5) and free of excess carbonates or alkali. Six pots were grown for each treatment.

The data obtained by plant analysis at the various harvest stages are given in Table 2 and Figure 1.

These data indicate the greater availability of the phosphorus when applied in the form of mono-ammonium phosphate as compared to mono-calcium phosphate and tricalcium phosphate. In every case, the pots treated with ammonium phosphate show a significantly higher uptake of phosphorus by the wheat plant than for the mono-calcium phosphate, or the tri-calcium phosphate treatments. Tri-calcium phosphate is recognized as not being an efficient type of phosphate carrier for neutral or alkaline soils, and its low availability in this experiment serves to emphasize such a conclusion.

The comparison of results as between ammonium phosphate and mono-calcium phosphate is the most significant from the practical standpoint. The data indicate that the calcium salt has a lower availability to the plant than does the ammonium phosphate, and it appears that the previously mentioned superiority of the ammonium phosphate in field performance can be ascribed to the greater availability of the phosphorus rather than to a direct beneficial effect of nitrogen as a nutrient. There is, however, the possibility of nitrogen stimulating the plant to a higher uptake of phosphorus. Further work on this point is in progress.

At present in Saskatchewan, there is only a small tonnage of fertilizer other than ammonium phosphate used on cereals. The data in this experiment indicate that phosphate applied as ammonium phosphate is likely to prove more effective per unit of phosphorus than it would if applied in the various forms of superphosphates. This test of availability demonstrates the particular value of the tracer technique in soil chemistry and plant nutrition. The statistical analysis indicates significant differences between the amounts of fertilizer phosphorus taken up from each fertilizer at all harvest dates, while only the larger differences are significant when total phosphorus values are compared.

Field tests in progress during the summer of 1948 appear to confirm the results of these greenhouse experiments and indicate that a test such as described above may prove useful in evaluating and comparing the efficiency of the different carriers of phosphorus without resorting to extensive field trials.

SUMMARY

Further greenhouse experiments are reported using radiophosphorus in fertilizer trials on wheat. It is shown that applications of phosphorus at seeding give the best response in growth although the uptake of fertilizer phosphorus may be increased by applying the material at two or four weeks after seeding. A further experiment confirms previous observations that mono-ammonium phosphate is a particularly suitable carrier of phosphorus for the generally base-saturated soils of this region. There was

a significantly greater uptake of phosphorus from mono-ammonium phosphate as compared to mono-calcium or tri-calcium phosphates at each of three periods of growth at which the wheat was harvested. It is suggested that such a test may be useful in evaluating the availability of various carriers of phosphorus when applied to different soils.

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THE NUTRITIVE VALUE OF NITROGENOUS COMPOUNDS FOR RUMINANTS

I. THE NUTRITIVE VALUE OF UREA AS A PROTEIN SUPPLEMENT[1]

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Since the beginning of the present century numerous experiments have been carried out in Germany to determine whether or not urea and other non-protein nitrogenous substances could be used as a source of nitrogen for the nutrition of ruminants. The results were not conclusive. In more recent years considerable interest has been aroused in the United States regarding possible uses of urea in feed stuffs. The results from many investigations have been sufficiently encouraging that recommendations have been made concerning the incorporation of urea in commercial feed stuffs. For instance, the Association of American Feed Control Officials, Inc., (1) states that urea may partially replace the nitrogen of protein concentrates up to 3 per cent of the concentrates. In the same way, the Quebec Provincial Feed Board in Canada (3), has made similar recommendations.

In this paper further information has been obtained on the use of urea for ruminant feeding. No literature review is included since that has been done by other authors in papers and reviews.

In the determination of the nutritive values of nitrogenous compounds for ruminants, a number of different methods have been used. These fall into two general classes—feeding trials and metabolism or balance trials. For small laboratory animals, a technique involving carcass analysis has been used to determine the increase in nitrogen or other body nutrients resulting from a specific dietary regime. Representative animals are slaughtered at the beginning of a feeding trial and analysed. The chemical composition is assumed to represent that of the remaining animals which are slaughtered and analysed at the end of the dietary treatment. The differences between the quantities of nutrients in the animal body at the beginning and end of the trial are assumed to represent the result of the feeding system used. This technique has been applied, in conjunction with feeding trials, to large animals in the present paper.

For this experiment 30 head of grade Shorthorn beef calves, weighing approximately from 150 to 200 kilograms, and 60 sheep, weighing approximately from 20 to 35 kilograms, were used. The animals were divided as uniformly as possible into groups of five each. From each group at the beginning of the experiment, two were slaughtered and analysed. Of the remaining three, one constituted the negative control receiving a nitrogen-poor basal ration. One was the positive control receiving the same basal ration with the addition of casein to supply the nitrogen requirements of the animals. This was accompanied by the simultaneous removal of starch or oil from the ration to balance the total digestible nutrients of the casein.

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[3] Senior Agricultural Assistants.

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The third animal was the experimental one receiving the basal ration with the addition of sufficient urea to equal the casein in content of digestible nitrogen. In each group the consumption of T.D.N. was equalized for the three animals. The quantities of T.D.N. given were based on the weights of the experimental urea animals in each group and estimated from Morrison's standards. Digestibility coefficients of the feeds, with the exception of molasses, were determined from digestion trials by this Division. Values for molasses were taken from Morrison's tables.

Part I. Beef Calves

Procedure

For the experiment with beef calves the basal, nitrogen-poor ration consisted of timothy hay, oat straw, barley, corn starch, corn oil and molasses. A typical ration is shown in Table 1. In addition the animals received mineral and vitamin supplements to satisfy their requirements.

TABLE 1.—TYPE OF BASAL NITROGEN-POOR RATION FED TO BEEF CATTLE

(Based on average of all basal rations fed to all groups)

Feed	Per cent in ration
Timothy hay	27.0
Oat straw	22.0
Barley	8.5
Corn starch	17.5
Corn oil	7.5
Molasses	17.5

The actual proportions of the feeds varied from group to group according to the individual tastes of the animals. While this ration was not free from nitrogen, the latter was well below the maintenance requirements. This will be shown later.

At the beginning of a trial for any one group the basal ration was fed to all animals until the live weights reached a constant level. Two of each group were then killed and analysed and to the remaining three were allotted the basal ration, basal ration plus urea or basal ration with casein. The allotment was, as far as possible, at random. In some cases, animals which gave indications of being poor eaters for the basal ration were chosen for slaughter. In one case a choice was made of the negative control animal.

Following the initial slaughter the animals were weighed weekly and the necessary adjustments made in the quantities of T.D.N. and digestible nitrogen. Feeds were analysed for dry matter and nitrogen throughout the experiment. At the end of about 40 to 50 weeks of feeding, the remaining three animals in the group were slaughtered and analysed. Slaughter weights and initial "empty" live weights were made after a twenty-four hour fast and a twelve-hour deprivation of water. The animals were clipped before weighing.

FIGURE 1. Group "A" just prior to slaughter.
No. 1 (*left*)—Negative control.
No. 2 (*middle*)—Urea.
No. 3 (*right*)—Positive control.

The slaughter technique was carried out in the following manner. The animals were fasted for 24 hours, clipped and killed. The clipped hair was discarded in the case of the two animals slaughtered in each group at the beginning of the trial. For the remaining three animals, slaughtered at the end of the trial, it was weighed and analysed. The blood was removed through the jugular vein, weighed and analysed for dry matter, nitrogen and ash. The hide, the empty washed digestive tract, the liver and the kidneys, were weighed and analysed for dry matter, nitrogen, fat and ash. The carcass was split into halves. One-half was separated into bone and meat. Each of these portions was weighed, sampled and analysed. The head, hoofs and tail-bone were treated as one sample. A final sample called fat and tissue consisted of extraneous fat, flesh and fat from the head, and the tongue. All samples were cooled with dry ice. The compilation of all these results gave the total composition of the entire animal expressed as per cent of the "empty" live weight.

Results

The six groups of steers were numbered A to F, respectively. With groups A, C, D and E, the experiment progressed without any serious incident. In group B, the negative control died. No obvious cause was revealed by a post mortem. In group F, the experimental urea animal died near the end of the trial. It was not possible to carry out any carcass analyses and the criterion for the value of urea was based on live weight gains only. A post mortem revealed no obvious cause of death. In some groups, for short periods, it was necessary to increase the proportion of hay in the ration at the expense of some or all of the other constituents. This was done when one or more animals in a group gave indications of going off feed. Apart from this the animals remained in good health and maintained their appetites. Figure 1 illustrates group A just prior to slaughter. No. 1 was the negative control, No. 2 the urea animal and No. 3 the casein animal.

The relationship of the digestible nutrients consumed to those required is given in Table 2.

The amount consumed is the mean daily quantity throughout the trial. The amount required is based upon the mean of the initial and final weights of the animals. It will be observed that the negative control animal received more than its requirements of T.D.N. and approximately one-half to two-thirds of its maintenance requirements of digestible protein.

TABLE 2.—COMPARISON OF DAILY NUTRIENTS CONSUMED WITH DAILY NUTRIENTS REQUIRED BASED ON MEAN LIVE WEIGHTS OF ANIMALS AND MORRISON'S TABLES. BEEF ANIMALS.

Group No.	Animal No.	Ration*	Daily values in kilograms				
			T.D.N.		D.C.P.		
			Required	Consumed	Required	Consumed	Maintenance requirement
A	14K	Neg. control	3.60–4.02	3.76	0.450–0.502	0.080	0.168
	39K	Urea	3.90–4.40	3.79	0.472–0.532	0.454	0.181
	36K	Pos. control	4.05–4.65	3.81	0.448–0.548	0.420	0.191
B	33H	Urea	3.28–3.63	2.99	0.420–0.472	0.297	0.150
	34H	Pos. control	3.45–3.83	3.01	0.439–0.489	0.313	0.160
C	35H	Neg. control	3.37–3.75	3.42	0.430–0.481	0.100	0.152
	36H	Urea	3.61–4.07	3.43	0.450–0.503	0.404	0.169
	37H	Pos. control	3.83–4.33	3.41	0.468–0.528	0.400	0.180
D	40H	Neg. control	3.12–3.48	3.25	0.403–0.458	0.098	0.140
	43H	Urea	3.39–3.76	3.25	0.432–0.482	0.380	0.154
	44H	Pos. control	3.80–4.38	3.25	0.463–0.523	0.377	0.178
E	48H	Neg. control	3.40–3.79	3.43	0.433–0.483	0.098	0.156
	46H	Urea	3.64–4.09	3.42	0.453–0.508	0.399	0.169
	47H	Pos. control	3.90–4.40	3.44	0.472–0.533	0.399	0.182
F	78503	Neg. control	3.00–3.30	3.00	0.391–0.442	0.095	0.131
	78495	Urea	3.08–3.42	3.00	0.400–0.452	0.315	0.138
	78507	Pos. control	3.20–3.55	2.99	0.412–0.464	0.295	0.143

* Negative control is nitrogen-poor ration.
Urea is the same ration with urea added.
Positive control is the casein ration.

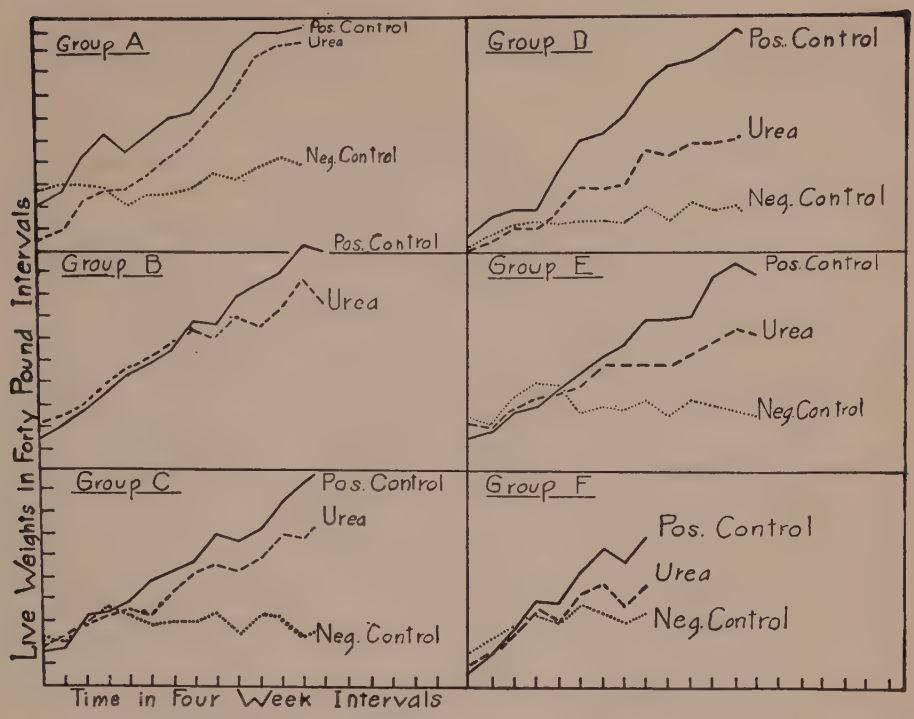

FIGURE 2. Weight increases of beef calves in the six groups.

The other two animals received amounts of T.D.N. and digestible crude protein closely approximating their requirements. In calculating the protein of the rations, the urea nitrogen was converted to its equivalent of digestible protein.

The weight changes are illustrated in Figure 2 at four-week intervals. It will be noted that the negative controls showed little change in live weight. In groups B, C, D, E and F the curve for the urea animals did not reach as high a level as that for the positive control. In group A the curves for the urea and the positive control were roughly parallel.

In Table 3 are summarized the gains in live weights and also the gains in body nutrients. Compared with the negative controls, the urea animals showed marked increases in the live weights and in the protein, fat and ash. Compared with the casein animals, with the exception of group A, the urea animal made less gains in live weight and body nutrients than the positive controls. In group A, the urea animal made a greater gain in live weight, weight of protein and weight of fat than the positive control but a smaller gain in ash.

The data for the urea and casein animals were analysed statistically. The method of paired values as described by Fisher (2) was used. The "t" values have been placed at the foot of the table. The differences between the two rations were significant for live weights and weights of protein and ash but not for fat.

In Table 4 are given the gains in total weight of carcass, and in the weights of individual carcass constituents. The urea animals compared

TABLE 3.—GAINS IN "EMPTY" LIVE WEIGHT AND BODY COMPONENTS. BEEF CALVES.

Group	Ration	Animal No.	Gains in kilograms			
			Empty live weights	Individual components		
				Protein (N × 6.25)	Fat	Ash
A	Neg. control†	14K	25	−1.2	21.5	−1.0
	Urea	39K	161	25.2	23.3	3.4
	Pos. control‡	36K	149	23.4	14.6	5.7
B	Urea	33H	120	21.4	13.8	4.3
	Pos. control†	34H	179	32.4	26.5	8.0
C	Neg. control†	35H	−6	−0.4	4.4	−0.9
	Urea	36H	65	13.9	22.3	2.0
	Pos. control‡	37H	128	24.7	31.6	4.6
D	Neg. control†	40H	28	6.7	13.9	1.6
	Urea	43H	92	14.5	38.0	1.0
	Pos. control‡	44H	145	27.8	38.6	4.0
E	Neg. control†	48H	−6	−0.1	11.8	−0.4
	Urea	46H	74	12.6	28.7	1.7
	Pos. control‡	47H	136	23.8	28.9	5.6
F	Neg. control†	78503	40	*	*	*
	Urea	78495	80	*	*	*
	Pos. control‡	78507	112	*	*	*
Means	Neg. control†		16	1.2	12.9	−0.2
	Urea		99	17.5	25.2	2.5
	Pos. control‡		142	26.4	28.0	5.6
"t" values for paired differences between urea and casein rations			3.59	3.28	0.75	10.06
Nec. "t" at P = 0.05			2.57	2.78	2.78	2.78

* Not determined for reasons given in text.
† Negative control—low nitrogen ration.
‡ Positive control—casein ration.

with the negative controls showed substantial gains. Their results were compared with those for the casein animals. The latter, with the exception of group A, had greater gains. Statistically, the gains in weights of total carcass, protein, ash and water of the casein animals were greater than those for the urea animals. The differences in fat gains were not significant.

In Table 5 are given the chemical compositions of the gains in the carcasses of the urea and casein animals. The "t" values are given at the foot of the table. Numerically, the percentages of protein, ash and water were higher for the casein animals, while the percentage of fat was lower. The differences for the protein and ash were statistically significant. While the differences in fat and water were not statistically significant, yet in groups A, C, D and E, the fat per cent was definitely higher in the urea animal with the water per cent lower. In group B the fat of the urea animal was quite low and the water correspondingly quite high. It can be concluded that the carcasses of the casein animals tended to have a higher percentage of protein and ash while the carcasses of the urea animals tended to be higher in fat and correspondingly lower in water.

TABLE 4.—GAINS IN WEIGHTS OF TOTAL CARCASSES AND OF INDIVIDUAL COMPONENTS. BEEF CALVES.

Group No.	Ration	Animal No.	Gains in kilograms					
			Total* carcass	Protein (N × 6.25)	Fat	Ash	Water	Sum of components
A	Neg. control	14K	9.2	−0.3	15.7	1.7	−3.3	13.8
	Urea	39K	83.2	15.4	16.7	2.1	48.3	82.5
	Pos. control	36K	74.8	15.1	10.9	3.4	45.9	75.3
B	Urea	33H	76.2	14.2	9.1	3.3	51.0	77.6
	Pos. control	34H	109.8	21.8	20.2	6.1	64.6	112.6
C	Neg. control	35H	4.0	−0.4	2.3	−0.9	5.0	6.0
	Urea	36H	48.0	8.1	13.8	1.7	28.2	51.8
	Pos. control	37H	89.1	16.8	18.3	3.4	54.4	92.9
D	Neg. control	40H	26.2	4.1	9.4	1.6	11.1	26.2
	Urea	43H	55.0	8.3	23.0	1.1	26.8	59.2
	Pos. control	44H	96.5	16.8	25.9	3.3	52.4	98.4
E	Neg. control	48H	2.1	−0.6	7.2	−0.7	−2.6	3.3
	Urea	46H	40.2	7.4	16.9	1.2	15.3	40.8
	Pos. control	47H	76.2	15.2	17.9	4.4	42.0	79.5
Means	Neg. control		10.4	0.7	8.6	0.4	2.6	12.3
	Urea		60.5	10.7	15.9	1.9	33.9	62.4
	Pos. control		89.3	17.1	18.6	4.1	51.8	91.7
"t" values for paired differences between urea and casein rations			3.06	3.80	1.00	6.46	3.18	—
Nec. "t" at P = 0.05			2.78	2.78	2.78	2.78	2.78	—

* Values for negative control will not be in line with gains in live weights in Table 3 due to (1) small gains or losses of live weights in negative controls, and (2) resulting magnification of error caused by an assumed original composition.

DISCUSSION

It is, therefore, evident that the animals receiving urea utilized it for the production of meat. It was not utilized as well as casein. In practice urea would not be fed as the sole source of nitrogen. Its value in this experiment relative to casein would not necessarily be the same as its relative value when incorporated into a mixed protein ration. In a further series of trials this point is being investigated.

Part II. Sheep

The procedure with sheep was similar to that used for steers, except that separate consideration was given to the nitrogen in the wool growth. The sheep were sheared prior to the experiment and prior to slaughtering. The basal ration was slightly different from that fed to the steers. Its type is given in Table 6.

It was composed of oat straw, turnips, apple pomace, flax bolls, oat hulls, corn meal, corn starch and corn oil, together with mineral and vitamin supplements.

TABLE 5.—CHEMICAL COMPOSITION OF GAINS IN CARCASSES OF ANIMALS ON UREA AND CASEIN RATIONS. BEEF CALVES.

Group No.	Ration*	Animal No.	Chemical composition of gains				
			Protein (N × 6.25)	Fat	Ash	Water	Sum of per cent
			%	%	%	%	
A	Urea	39K	18.6	20.0	2.4	58.1	99.1
	Casein	36K	20.1	14.6	4.6	61.4	100.7
B	Urea	33H	18.7	12.0	4.4	67.0	102.1
	Casein	34H	19.8	18.4	5.5	58.8	102.5
C	Urea	36H	16.9	28.8	3.6	58.9	108.2
	Casein	37H	18.8	20.5	3.8	61.0	104.1
D	Urea	43H	15.1	41.8	2.0	48.7	107.6
	Casein	44H	17.4	26.9	3.4	54.3	102.0
E	Urea	46H	18.5	42.1	3.0	38.0	101.6
	Casein	47H	19.9	23.4	5.8	55.1	104.2
Means	Urea	—	17.6	28.9	3.1	54.1	103.7
	Casein	—	19.2	20.8	4.6	58.1	102.7
Difference			+1.6	−8.1	+1.5	+4.0	—
"t" values (Nec. "t" at P = 0.05 is 2.78)			7.85	1.89	3.45	0.98	—

* Casein animals—positive controls.

This experiment was unsatisfactory. It was impossible to induce the sheep to eat much more than the maintenance requirements. The result was that with the exception of a few cases where casein was fed, the animals showed losses in weight and body constituents. Criteria, under these circumstances, would be firstly, the ability of different rations to prevent losses in body nutrients and secondly, the amount of protein laid down in the wool growth. These are of limited significance. The results support those with steers and have, therefore, been recorded.

During the progress of the experiment, one whole group and the negative control in a second group had to be removed from the experiment. This was due either to failure in food consumption or death. There were

TABLE 6.—TYPE OF BASAL RATION FED SHEEP

(Based on daily mean of all rations for all animals)

Feed	Per cent in ration
Oat straw	25
Turnips	42
Apple pomace	5
Flax bolls	2
Oat hulls	6
Corn meal	6
Corn starch	10
Corn oil	4

left, therefore, 10 groups of 3 each and one group of two animals—the urea and the positive control. These have been numbered 1 to 11 in the experiment.

In Table 7 are given the mean daily consumption of T.D.N. and digestible protein compared with Morrison's standards for growing and fattening sheep. The weights of the animals were the average of the initial

TABLE 7.—COMPARISON OF DAILY NUTRIENTS CONSUMED WITH DAILY REQUIREMENTS BASED ON MEAN LIVE WEIGHTS OF ANIMALS AND MORRISON'S TABLES. SHEEP.

Group No.	Animal No.	Ration	Daily values in kilograms					
			T.D.N.			D.C.P.		
			Required		Consumed	Required		Consumed
			Maintenance	Growth		Maintenance	Growth	
1	119R	Neg. control	0.39	0.67	0.48	0.04	0.09	0.02
	12R	Urea	0.39	0.68	0.54	0.04	0.09	0.18
	129R	Pos. control	0.43	0.78	0.57	0.04	0.09	0.08
2	127R	Neg. control	0.51	1.03	0.49	0.04	0.10	0.02
	26R	Urea	0.48	0.94	0.54	0.04	0.10	0.19
	103R	Pos. control	0.50	0.98	0.58	0.04	0.10	0.09
3	110R	Neg. control	0.46	0.87	0.49	0.04	0.10	0.02
	143R	Urea	0.44	0.83	0.55	0.04	0.10	0.20
	134R	Pos. control	0.48	0.94	0.58	0.04	0.10	0.09
4	20S	Neg. control	0.45	0.79	0.34	0.04	0.09	0.01
	122S	Urea	0.45	0.84	0.45	0.04	0.10	0.08
	46S	Pos. control	0.44	0.81	0.45	0.04	0.09	0.08
5	135S	Neg. control	0.41	0.71	0.30	0.04	0.09	0.01
	44S	Urea	0.41	0.72	0.39	0.04	0.09	0.07
	21S	Pos. control	0.43	0.80	0.44	0.04	0.09	0.07
6	47S	Neg. control	0.40	0.67	0.43	0.04	0.09	0.01
	126S	Urea	0.43	0.78	0.45	0.04	0.09	0.08
	154S	Pos. control	0.41	0.73	0.44	0.04	0.09	0.08
7	94T	Neg. control	0.36	0.57	0.28	0.04	0.08	0.01
	91T	Urea	0.34	0.50	0.28	0.04	0.07	0.06
	123T	Pos. control	0.38	0.64	0.28	0.04	0.09	0.07
8	102T	Neg. control	0.36	0.58	0.33	0.04	0.08	0.01
	116T	Urea	0.37	0.62	0.33	0.04	0.08	0.07
	99T	Pos. control	0.40	0.70	0.32	0.04	0.09	0.08
9	104T	Neg. control	0.34	0.52	0.30	0.04	0.07	0.00*
	42T	Urea	0.36	0.58	0.29	0.04	0.08	0.05
	119T	Pos. control	0.36	0.57	0.29	0.04	0.08	0.06
10	30T	Neg. control	0.33	0.50	0.27	0.03	0.07	0.00*
	699T	Urea	0.31	0.48	0.26	0.03	0.06	0.05
	112T	Pos. control	0.34	0.53	0.26	0.04	0.07	0.06
11	37T	Urea	0.40	0.69	0.30	0.04	0.09	0.06
	85T	Pos. control	0.39	0.66	0.30	0.04	0.09	0.07

* The ration was not completely free from nitrogen.

TABLE 8.—CHANGES IN "EMPTY" LIVE WEIGHT AND BODY NUTRIENTS, AND GAINS IN WOOL NITROGEN. SHEEP.

Group No.	Animal No.	Ration	Changes in weights			Gain in wool nitrogen, gm.
			Live weight, kg.	Protein (N × 6.25), gm.	Fat, kg.	
1	119R	Neg. control	− 7	− 700	− 2.7	45
	12R	Urea	− 5	− 119	− 2.6	64
	129R	Pos. control	− 2	+ 238	− 2.6	61
2	127R	Neg. control	− 7	− 956	+ 0.6	44
	26R	Urea	−10	− 856	− 2.7	86
	103R	Pos. control	− 8	− 56	− 3.0	91
3	110R	Neg. control	− 6	− 856	− 3.5	58
	145R	Urea	− 5	− 569	− 1.6	65
	134R	Pos. control	− 2	+ 219	− 3.9	113
4	20S	Neg. control	− 7	− 625	− 2.4	41
	122S	Urea	− 7	− 781	− 1.7	61
	46S	Pos. control	− 6	− 212	− 3.3	65
5	135S	Neg. control	−10	−1419	− 1.7	37
	44S	Urea	− 3	− 225	− 0.5	49
	21S	Pos. control	− 5	− 306	− 1.3	87
6	47S	Neg. control	− 5	−1069	− 3.2	40
	126S	Urea	− 5	− 469	− 2.3	80
	154S	Pos. control	− 5	− 250	− 3.6	106
7	94T	Neg. control	− 1	+ 262	− 3.3	26
	91T	Urea	0	− 44	− 3.1	22
	123T	Pos. control	+ 4	+ 506	− 1.6	57
8	102T	Neg. control	− 3	− 550	− 3.4	22
	116T	Urea	0	− 6	− 3.7	60
	99T	Pos. control	+ 5	+ 525	− 3.1	80
9	104T	Neg. control	− 4	− 675	− 2.7	25
	42T	Urea	− 2	− 306	− 2.4	33
	119T	Pos. control	− 2	− 338	− 2.9	25
10	30T	Neg. control	− 2	− 800	− 1.1	23
	699T	Urea	− 3	− 781	− 1.6	27
	112T	Pos. control	+ 2	− 362	− 1.3	35
11	37T	Urea	− 2	− 400	− 4.2	44
	85T	Pos. control	+ 2	+ 156	− 3.9	38

and final weights. As far as the T.D.N. were concerned the amount consumed was approximately the same as the maintenance requirements and much less than the growth requirements. In five groups the negative controls consumed somewhat less than the others. In regard to the digestible protein the negative control consumed amounts which were less than half the maintenance requirements. This was according to plan. In the first three groups, urea was added at such a rate that the nitrogen was twice that given in the casein. In the remaining groups they were fed

on an equivalent digestible nitrogen basis. The nitrogen consumed from the urea and casein rations approached the requirements for growing and fattening sheep.

In Table 8 are given the amounts of nitrogen put down in the wool, and the changes in live weights, body protein and body fat. The casein ration produced more nitrogen in the wool than the urea ration. The latter, in turn, produced more than the negative controls. These differences, when considered as paired values, were statistically significant.

SUMMARY AND CONCLUSION

1. The value of urea as a source of protein for ruminants was investigated by means of feeding and slaughter trials with 30 head of beef calves and 60 lambs.

2. The animals were divided into groups of five each, two of which were killed and analysed at the commencement of the feeding trial and the remaining three killed and analysed at the end of the trial.

3. Of the three remaining animals in each group, one received a nitrogen-poor basal ration, one received the same ration with urea and one received the same ration with casein equal in digestible nitrogen content to the urea, with sufficient starch or oil removed to equalize the T.D.N. content.

4. The weights and compositions of the three animals in each group at the end of the trial were compared with the initial weights and the initial compositions estimated from the two slaughtered animals.

5. In the case of the beef calves, the animals receiving the nitrogen-poor ration, while maintaining good health and appetite, made small live weight gains, showed practically no deposition of protein or ash but did show some increase in fat.

6. The beef calves receiving the urea ration made relatively good gains in live weight and body nutrients.

7. The beef calves receiving the casein rations made appreciably greater gains in live weight, body protein and ash than those receiving the urea. The gains in body fat were of a similar order for both rations.

8. The gains in total weights of the carcasses, and the gains in weights of protein, ash and water in these carcasses, were greater for the beef calves receiving casein than for those receiving urea. There was little difference between the gains in fat in the carcass with these two rations.

9. The beef calves receiving the nitrogen-poor ration showed some gains in carcass weight and carcass fat with negligible gains in carcass protein and water.

10. As far as chemical composition of carcass gains was concerned those from the beef calves receiving casein tended to be higher in protein, ash and water and lower in fat than those from the animals receiving urea.

11. With the sheep, the casein ration produced more wool nitrogen than the urea and the latter more than the nitrogen-poor ration.

12. Due to the failure of the sheep to consume much in excess of their maintenance requirements for T.D.N., satisfactory conclusions of the value of urea for meat production could not be drawn. The casein rations maintained the body protein against losses to a greater degree than the urea ration and the latter to a greater degree than the nitrogen-poor ration.

13. It was concluded that under the conditions of these experiments and with the rations employed, urea acted as a substitute for feed protein. Its value was less than that of casein.

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THE NUTRITIVE VALUE OF NITROGENOUS COMPOUNDS FOR RUMINANTS

II. THE FORMATION OF BODY PROTEIN FROM UREA LABELLED WITH THE ISOTOPE N^{15}.[1]

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In the first paper of this series (8) it was shown by means of carcass analyses that animals receiving urea deposited more protein than animals receiving the basal ration. In both cases the basal ration was nitrogen-poor. It was not entirely free from nitrogen. There were three possible explanations for the increased protein deposition. The nitrogen of the urea could have been actually used to form protein through the agencies of the microflora of the paunch. The presence of urea may have increased the activity of the microflora to the extent that they would have a greater dissolution action on the fibre of roughages. This could result in the release of more nitrogen for the animal body. Finally, nitrogen of urea may have been used by the microflora as a source of nitrogen in place of the feed nitrogen.

To investigate this point, urea labelled with N^{15} was fed to sheep over a 4-day period. The animals were killed. A sample of oxalated blood was taken. The liver and kidneys were removed and frozen with dry ice. The blood was centrifuged and the plasma used for nitrogen determinations. Trichloracetic acid was used to precipitate the proteins in the plasma and to separate the proteins from the non-proteins in the liver and kidneys. The N^{15} was determined in the non-protein and protein fractions. It was found that in the blood, the kidneys and the livers the protein contained N^{15} in excess of that which would be normally present in the animal body. This fact, combined with the results of the previous paper (8), is considered to be evidence that the nitrogen of the urea is utilized by ruminants to form body proteins.

The principles of the methods of separation and analyses were based on the papers of R. Schoenheimer, G. L. Foster, D. Rittenberg and S. Ratner (4), R. Schoenheimer and D. Rittenberg (7), D. Rittenberg, A. S. Keston, F. Rosebury and R. Schoenheimer (3), A. S. Keston, D. Rittenberg and R. Schoenheimer (1), R. Schoenheimer, S. Ratner and D. Rittenberg (5) and R. Schoenheimer, S. Ratner and D. Rittenberg (6).

EXPERIMENTAL

1. *Preparation of urea containing N^{15}.* The method of preparation is given in the third paper of the series by Leitch and Davidson (2).

2. *Separation of protein and non-protein nitrogen.* The separation of the protein from non-protein nitrogen was effected by the use of trichloracetic acid. In the case of the blood, 100 ml. of 6 per cent trichloracetic were added to 10 ml. of plasma. It was allowed to stand for several hours, decanted on to a filter paper and the procedure repeated twice more. The precipitate was then transferred completely and thoroughly washed. In

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TABLE 1.—N^{15} CONCENTRATION OF TISSUES OF SHEEP RECEIVING NORMAL UREA

Sheep No.	Fraction	N^{15} concentration* (atom per cent excess)
18B	Liver protein N	− 0.040
	Liver NPN	− 0.043
	Blood plasma protein N	+ 0.002
	Blood plasma ammoniacal + urea N	− 0.006
	Urine ammoniacal + urea N	+ 0.016
51B	Liver, protein N	+ 0.011
	Blood plasma protein N	− 0.001
	Kidney protein nitrogen	+ 0.006
	Sample of normal urea	+ 0.003

* Normal abundance of Washington air 0.363 atom per cent.

TABLE 2.—N^{15} CONCENTRATION OF TISSUES OF SHEEP RECEIVING UREA LABELLED WITH N^{15}

Sheep No.	Fraction	N^{15} concentration* (atom per cent excess)
88B	Liver protein N	5.141
	Liver NPN	0.861
	Urine ammoniacal + urea N	2.722
	Urine ammoniacal + urea N	2.429
	Urine ammoniacal N	− 0.005
	Urine ammoniacal N	+ 0.826
29B	Liver protein N	0.502
	Liver NPN	1.314
	Blood plasma protein N	0.153
	Blood plasma NPN	1.872
	Blood plasma ammoniacal + urea N	4.787
	Kidney protein N	0.314
	Kidney NPN	0.658
86B	Liver protein N	0.296
	Liver protein N	0.307
	Liver NPN	0.867
	Blood plasma protein N	0.074
	Blood plasma NPN	1.347
	Kidney protein N	0.232
	Kidney protein N	0.236
	Kidney NPN	1.650
	Sample of urea labelled with N^{15}	31.767

* Normal abundance of Washington air 0.368 atom per cent.

the case of the frozen liver and kidneys 10 gram samples were triturated in an iron mortar with 100 ml. of 6 per cent trichloracetic acid. The material was ground to a homogenous pulp. It was then transferred to a beaker and the procedure mentioned above for blood carried out.

3. *Method of Determination of* N^{15}. The various materials were digested by the Kjeldahl procedure for approximately 12 hours using mercury as a catalyst. After the ammonia had been liberated from an

aliquot in a micro Kjeldahl distillation apparatus, the nitrogen in the resulting ammonium chloride was liberated with alkaline hypobromite according to the method of Rittenberg *et al* (3). The apparatus used was the same as Rittenberg's with a few modifications. The N^{15} concentrations in the resulting samples of nitrogen gas were determined with the mass spectrometer by the Bureau of Standards, Washington.

4. *Rations and animals.* The animals used were Leicester-Shropshire sheep weighing approximately 35-40 Kg. The basal ration consisted of:

Straw	50
Starch	100
Corn oil	20
Oats	100
Hay	600

The animals were fed as much as they could consume but for each pair the consumption was equalized. Vitamins and minerals were fed. Three pairs of animals were used, numbered Groups A, B and C. Within each pair, one animal received normal urea and one received urea containing approximately 30 atom per cent N^{15}. The groups received in 4 days approximately 10 to 12 grams of urea or isotopic urea. This was given mixed with calcium sulphate as a filler in six gelatin capsules daily. The administration was at 4:00 in the afternoon and 9:00 in the morning just following the feeding of the basal ration. On the last day the animals were killed at 1:00 o'clock and the samples of blood, liver and kidneys analysed as described above.

5. *Results.* In Table 1 are given the N^{15} concentrations of various fractions of the animal receiving normal urea. In Table 2 are given the N^{15} concentrations for the animals receiving urea labelled with isotopic nitrogen. These are expressed in terms of atom per cent excess above normal abundance. It is quite evident from these tables that the N^{15} of the urea has been incorporated into the body proteins.

TABLE 3.—N^{15} CONCENTRATION OF PROTEIN NITROGEN AND NON-PROTEIN NITROGEN FRACTIONS OF LIVER TO WHICH N^{15} WAS ADDED

Sample No.	Substance added	Fraction	N^{15} atom per cent excess†
29	$N^*H_4NO_3$	Protein N	− 0.001
30	$N^*H_4NO_3$	Protein N	+ 0.024
9	$N^*H_4NO_3$	Protein N	+ 0.007
10	$N^*H_4NO_3$	Protein N	+ 0.011
11	Urea*	Protein N	+ 0.079
12	Urea*	Protein N	+ 0.024
32	$N^*H_4NO_3$	NPN	7.920
33	$N^*H_4NO_3$	NPN	15.594
34	$N^*H_4NO_3$	NPN	23.388
1	$N^*H_4NO_3$	NPN	14.659
2	$N^*H_4NO_3$	NPN	15.938
3	$N^*H_4NO_3$	NPN	13.385
4	$N^*H_4NO_3$	NPN	3.690
5	Urea*	NPN	7.015
6	Urea*	NPN	7.153

N* Containing approximately 30 atom per cent excess N^{15}.
† Normal abundance of N^{15} in Washington air 0.363, 0.369 and 0.370 according to date of determination.

6. *Testing method of separation.* In the interpretation of results of this type of work the question arises as to whether or not trichloracetic extraction effects a complete separation of protein nitrogen from non-protein nitrogen. To check this, samples of liver were ground in a mortar with varying amounts of ammonium nitrate and urea both of which contained N^{15}. In the case of the ammonium nitrate the concentration was approximately 30 atom per cent in the ammonium radical. In the case of urea the concentration was approximately 30 atom per cent. Separations of NPN and protein nitrogen were carried out by the method described above. The results for the N^{15} content of the protein and non-protein fraction are given in Table 3. This shows that trichloracetic acid was an effective agent for separating the non-protein from the protein material.

SUMMARY AND CONCLUSIONS

1. Urea containing approximately 30 atom per cent N^{15} was fed to sheep for 4 days.

2. The proteins separated from the liver, blood and kidneys contained N^{15} in excess of normal abundance.

3. It was concluded that nitrogen from urea was utilized by ruminants for the formation of body proteins.

ACKNOWLEDGMENTS

Acknowledgment is made to the Division of Animal Husbandry, Experimental Farm Service, for providing the experimental animals and carrying out the dosing with the gelatine capsules.

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THE NUTRITIVE VALUE OF NITROGENOUS COMPOUNDS FOR RUMINANTS

III. SYNTHESIS OF UREA CONTAINING N^{15}

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In the preceding paper of this series (1) the utilization of urea as a substitute for protein nitrogen in the metabolism of ruminants was confirmed with the aid of urea labelled with N^{15}. The purpose of this paper is to describe the synthesis of isotopic urea from ammonium nitrate containing N^{15} in the ammonium group.

The classic synthesis of Wöbler from potassium cyanate and ammonium nitrate was not applicable in this case because it leads to isotopic dilution of the N^{15}. In the commercial synthesis of urea from ammonia and carbon dioxide a large excess of ammonia is required to realize yields of the order of 80 per cent. Hentschel (2) reported the preparation of urea in 70 per cent yields by passing a rapid stream of dry ammonia through liquid diphenyl carbonate. For the present purpose this method has the same disadvantage as the commercial method. Besides, no urea could ever be isolated from the reaction mixture in our experience. Kirkhgof and Astrova (3) obtained 70 per cent yields of urea by heating a mixture of 22 per cent ammonia and diphenyl carbonate for two hours. The method finally chosen was based on that of these authors. Urea was obtained in yields close to 90 per cent by heating the reactants in a sealed tube at 100° C. for four hours. Directions for preparing urea from ammonium nitrate containing the isotope in the ammonium group are given in detail below.

EXPERIMENTAL

Apparatus

The apparatus is shown in the adjoining figure. "A" is a 100 ml. flask with a trap to prevent any material from spattering over into the reaction tube. "C" contains the diphenyl carbonate and water. The constriction at "F" is to allow easy sealing after the ammonia has distilled over. "D" is a manometer.

Materials

Diphenyl carbonate was prepared as directed by Hentschel (*loc. cit.*) from phosgene and phenol. The ammonium nitrate containing 32 atom per cent excess N^{15} was purchased from the Eastman Kodak Company.

Procedure

Twenty-five ml. of 50 per cent potassium hydroxide were placed in the separatory funnel "B". Eight grams of labelled ammonium nitrate were placed in the flask "A". The tube "C" contained 12.0 gm. of diphenyl carbonate and 8 ml. of water. It was then cooled in liquid air and the system was evacuated. With stop-cock "E" closed, alkali was added dropwise at such a rate that foaming into the trap was avoided. The ammonia generated collected in the reaction tube which was sealed off after an hour. There was practically no rise in pressure within the system during this time. The tube was allowed to come to room temperature and

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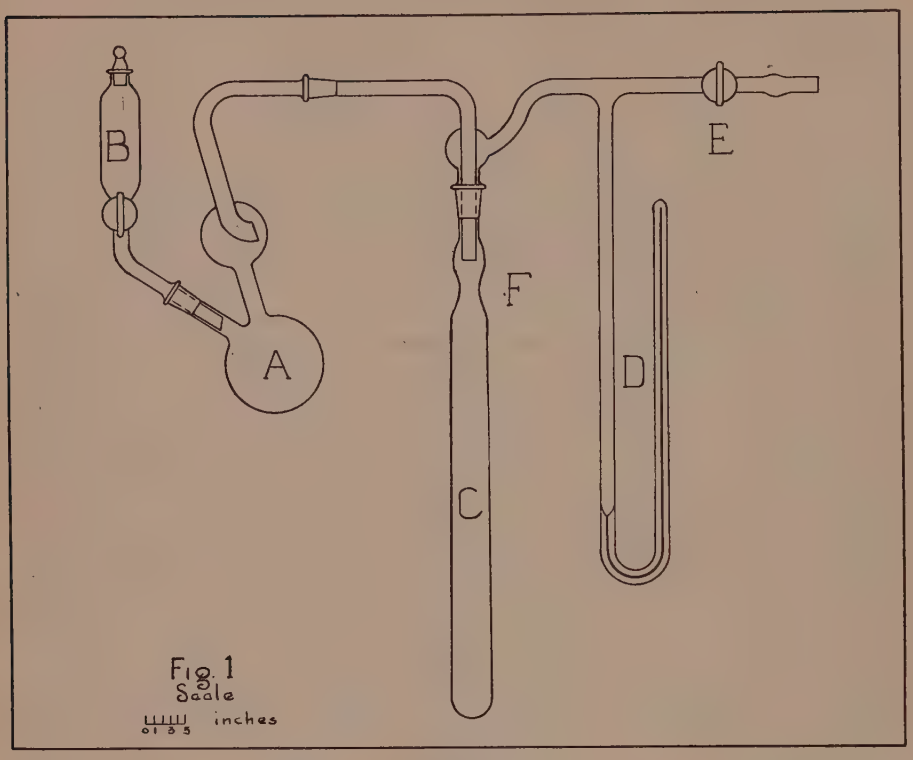

then heated for four hours in a water-bath at 100° C. A small amount of ammonia remaining in the system was absorbed in 10 per cent hydrochloric acid while warming the alkali in the generator.

The reaction products from five such runs were generally worked up together. The combined liquors were diluted to about 200 ml. with water and extracted twice with ether to remove phenol. Crude urea was recovered by evaporating the aqueous solution in vacuo and purified by recrystallization from absolute ethanol using carbon black. The total yield of urea, m.p. 131-3° C., from two crops was 13.0 gm. (86.6 per cent).

In order to recover N^{15} from the mother liquor it was evaporated to dryness in a Kjeldahl flask. The residue was digested with 15 ml. of concentrated sulphuric acid and a trace of copper sulphate. The ammonia liberated by adding 60 ml. of 50 per cent sodium hydroxide to the acid solution (previously diluted to about 150 ml. with water and then cooled) was distilled into the hydrochloric acid used earlier to recover ammonia in the generator. On evaporation to dryness 3.0 gm. of ammonium chloride were obtained. The yield after allowing for recovered ammonium chloride was nearly quantitative.

The synthesis of isotopic urea was also recently reported by Cavalieri, Blair and Brown (4).

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DINITROPHENOL DERIVATIVES AS SUMMER ACARICIDES IN BRITISH COLUMBIA[1]

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The insecticidal properties of dinitrophenol and a number of its derivatives have been known for many years but it was not until 1936 that their value as acaricides was recognized. That year Boyce and Prendergast (2) reported that 2,4-dinitro-o-cyclohexylphenol was highly toxic to the citrus red mite, *Paratetranychus citri* McG. Later, Boyce *et al.* (3, 4, 5) investigated dinitro-o-cyclohexylphenol with and without oil, and its sodium, nicotine, ammonium and triethanolamine salts. They concluded that the citrus red mite, the six-spotted mite, *Tetranychus sexmaculatus* Riley, the common red spider, *T. telarius* (L.) (sic) and the brown clover or almond mite, *Bryobia praetiosa* Koch could be satisfactorily controlled by a one per cent dust of dinitro-o-cyclohexylphenol. Morrison and Mote (10) and Simpson (11) reported excellent control of the common red spider on hops by the same compound at concentrations as low as 0.031 per cent and 0.025 per cent, respectively. Greenslade & Goscombe (7) obtained fair results against the European red mite, *Paratetranychus pilosus* (C. & F.), with one per cent dust of dinitro-o-cyclohexylphenol.

The dicyclohexylamine salt of 2,4-dinitro-o-cyclohexylphenol was first examined as an acaricide by Kagy and McCall (9) who found that, although its toxic action was slower on the citrus red mite than that of the parent compound, it was more persistent. They claimed that the salt was less phytotoxic than the parent compound and that a dust containing approximately 1.7 per cent of the salt was very toxic to the common red spider, the six-spotted mite, the European red mite, the clover mite and the Pacific mite, *Tetranychus pacificus* (McG.). Subsequently, *DN-111*, which is a commercial formulation containing 20 per cent of the dicyclohexylamine salt of dinitro-o-cyclohexylphenol, came into common use as a summer spray. Hough (8) compared several materials for European red mite and apple mite (*Tetranychus schoenei* McG.) control in DDT-sprayed plots. These included *DN-111* at the rate of 1.25 pounds and *DN-Dry Mix No. 1* (40 per cent dinitro-o-cyclohexylphenol) at 0.67 pound per 100 gallons. The latter gave best results. The same substance used at 6 ounces per 100 gallons as a summer spray gave good results according to Cutright and Sutton (6).

EXPERIMENTAL RESULTS

In British Columbia orchards the mite problem has become increasingly troublesome during the last ten years. As early as 1938 growers reported lack of control by materials which previously had appeared quite satisfactory. In 1939 the Dominion Entomological Laboratory, Summerland, initiated experiments with dinitrophenol derivatives for control of European red mite and Pacific mite. The first trials with these compounds

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included both dormant and summer applications of oil solutions of 3,5-dinitro-o-cresol and 2,4-dinitro-o-cyclohexylphenol for European red mite control. The addition of dinitro-o-cresol either to dormant oil or to summer oil in an amount tolerated by apple trees did not seem to improve control. Dinitro-o-cyclohexylphenol, on the other hand, appeared fairly effective in summer oil solution but caused a peculiar browning of leaf petioles and veins. In 1940 the same compounds in oil solution were tested as delayed dormant treatments for Pacific mite and proved to be unsatisfactory.

Summer experiments with the dicyclohexylamine salt of dinitro-o-cyclohexylphenol were undertaken in 1942. Two years' work with a dust (*DN-8*) containing 1.2 per cent of the salt and 1.8 per cent low viscosity oil led to the conclusion that it considerably reduced European red mite and Pacific mite populations but was probably less effective than either one per cent light to medium summer oil or a commercial derris-pine oil-petroleum spray.

With the introduction of DDT for codling moth control the mite problem was further intensified. Among other acaricides, one of the first commercial dinitrophenol derivatives to be examined was *DN-111*. In 1944, this product was used at the rate of one pound per 100 Imperial gallons of spray mixture in five cover sprays against the European red mite. The last spray was applied August 2 and records of the mite population at the end of the season, 34 days later, are summarized in Table 1.

TABLE 1.—EFFECT OF FIVE COVER SPRAYS OF DN-111, 1 LB. : 100 GAL. UPON THE POPULATION OF EUROPEAN RED MITES IN APPLE ORCHARDS (SEPT. 5-6, 1944)

Plot	Materials per 100 Imperial gallons		Average mites per leaf
A	Cryolite*	4 lb.	30.1
	Casein	0.5 oz.	
	Hydrated lime	4 oz.	
	DN-111	1 lb.	
B	Cryolite	4 lb.	25.7
	Casein	0.5 oz.	
	Hydrated lime	4 oz.	
C	DDT tech.	0.5 lb.	24.8
	Acetone	1 qt.	
	Sodium lauryl sulphate†	2 oz.	
	DN-111	1 lb.	
D	DDT tech.	0.5 lb.	59.5
	Acetone	1 qt.	
	Sodium lauryl sulphate	2 oz.	

* Alorco.
† Duponol W. A. Paste.

The addition of the dicyclohexylamine salt of dinitro-o-cyclohexylphenol 0.2 pound per 100 Imperial gallons in five cover sprays failed to reduce populations of the European red mite to a satisfactory level. Further, hydrated lime apparently lessened the effectiveness of the compound which, incidentally caused neither foliage nor fruit injury. The

following year *DN-111* was used with DDT or cryolite at 1.5 pound per 100 Imperial gallons in four cover sprays. Mite damage was assessed by a general inspection of the trees. There was little evident effect on abundance of European red mite but apparently good control of Pacific mite; no spray injury occurred. Where lime was used in the spray mixture, once again the effectiveness of the acaricide appeared to be reduced. Since 1945, *DN-111* has been used on a large scale by growers and to some extent in experimental work. In a number of cases results in European red mite control have been disappointing.

Prior to 1945, the Summerland laboratory had prepared the ammonium and monoethanolamine salts of dinitro-o-cyclohexylphenol. These two salts used in field trials as summer acaricides in 1945 and 1946, were compared with a number of other acaricides then in general use or under experiment. The basic formula was 2 ounces of technical dinitro-o-cyclohexylphenol and one ounce of either 28 per cent ammonia or technical monoethanolamine. Both salts gave satisfactory control of European red mite and Pacific mite. More detailed information of their effectiveness was obtained from hand sprayer (bucket pump) experiments in 1946. In these trials three gallons of each spray mixture were applied to branches of McIntosh apple trees heavily infested with European red mite. No spreading agent was added to the spray materials. Counts of living mites were made microscopically approximately one week after the spray date; egg mortality was not recorded. In Table 2 are given results adjusted according to Abbott's formula (1).

TABLE 2.—EFFECT OF HAND SPRAYER APPLICATIONS OF ACARICIDES UPON EUROPEAN RED MITES INFESTING MCINTOSH APPLE TREES (1946)

Materials per 100 Imperial gal.		Mites examined	Per cent living mites	Per cent control
Dinitro-o-cyclohexylphenol Monoethanolamine	2 oz. 1 oz.	1438	0.4	99.2
Dinitro-o-cyclohexylphenol Monoethanolamine	1 oz. 0.5 oz.	1277	12.5	84.0
Di-para-chlorophenylmethyl carbinol (25%)	2 lb. 0.5 lb.	536	0.9	98.9
	Trial A	840	1.7	97.4
	Trial B	432	7.2	92.0
DDT (16%) hydroxypentamethyl flavan (20%)*	5.0 lb. 2.5 lb.	1410 1008	1.2 3.3	97.7 93.9
Dinitro-o-cyclohexylphenol Ammonia (28%)	2 oz. Trial A 1 oz. Trial B	1139 770	4.0 13.5	94.8 84.8
Dicyclohexylamine dinitro-o-cyclohexylphenolate (20%)	1 lb. 0.5 lb.	987 955	5.6 24.0	91.2 62.3
Disodium ethylene bis dithiocarbamate**	2.0 lb. 0.5 lb.	576 663	49.3 59.6	44.6 33.0
DDT (25%), mixture alkyl naphthyl ethers (72%)†	2.0 lb. 0.5 lb.	789 656	65.4 80.6	26.5 9.4

* DDT Miticidal.
** Dithane D-14.
† Syndeet.

TABLE 3.—EFFECT UPON EUROPEAN RED MITES OF ACARICIDES INCLUDED IN THE FIRST FOUR COVER SPRAYS OF 50 PER CENT DDT 1 LB. : 100 GAL. AVERAGE NUMBER MITES PER LEAF*

Plot	Materials per 100 Imperial gallons	May 26	June 3	June 10	June 18	June 30	July 8	July 16	July 29	Aug. 11	Aug. 20	Sept. 11	Season average
A	Check Plot Cryolite **, 4 lb. Summer oil†, 0.25 gal. (1st and 5th covers), 0.5 gal. (2nd, 3rd and 4th covers)	0.9	5.0	8.1	1.6	—	19.4	42.7	15.8	13.9	5.4	1.6	11.4
B	Dinitro-o-cyclohexylphenol, 2 oz. Monoethanolamine, 1 oz.	0.1	0.2	0.3	0.2	—	6.1	14.8	12.4	22.0	8.9	2.9	6.8
C	Dinitro-o-cresol, 2 oz. Monoethanolamine, 1 oz.	1.1	19.1	17.5	7.9	75.6				Discontinued			
D	Dinitro-o-cresol (50%), 4 oz.	0.5	18.1	21.7	24.5	130.8				Discontinued			
E	Check Plot (same as Plot A)	0.4	1.5	2.3	0.9	—	8.0	44.1	13.2	12.2	1.9	3.3	8.8
F	Dicyclohexylamine dinitro-o-cyclo-hexylphenolate (20%), 0.5 lb.	0.6	7.8	7.2	7.9	—	48.9	59.6	39.5	11.5	10.8	1.1	19.5

* Vertical lines indicate spray treatments.
** Kryocide.
† Shell Helix 15.

In these trials no spray injury occurred. Control of European red mite by monoethanolamine dinitro-o-cyclohexylphenolate was apparently excellent; the ammonium salt seemed slightly less effective.

In 1947, fairly extensive experiments were undertaken with 20 acaricides in a number of combinations and dilutions. These included such dinitrophenol derivatives as 3,5-dinitro-o-cresol, 2,4-dinitro-o-cyclohexylphenol and 2,4-dinitro-6-secondary butyl phenol. In one series of experiments some of these compounds and their monoethanolamine and dicyclohexylamine salts were applied throughout the season for European red mite control in combination with DDT codling moth sprays; cryolite-oil was used throughout the season on the several check plots. A full spray program consisted of five codling moth sprays applied May 17, May 27, June 13, July 23 and August 13 by a stationary high pressure sprayer. The acaricide was included in the first four sprays but, as will be noted in Table 3, in two instances the mite populations rose to such high levels that the acaricides then in use had to be discontinued in favour of more toxic materials. Each plot consisted of 5 to 8 Delicious apple trees of which five trees were sampled for mites by the Venable's technique (12) before spraying (except before first cover) and again three or more days after spraying. Results are shown in Table 3.

In the same experimental block single applications of a number of acaricides were made at various times in June and July. Results from the dinitrophenol derivatives and their monoethanolamine salts are shown in Table 4.

TABLE 4.—COMPARISON OF MITICIDES IN SINGLE APPLICATIONS. AVERAGE NUMBER EUROPEAN RED MITES PER LEAF

Trial	Materials per 100 Imperial gal.	Spray date	Before spray	Days after spray					
				4	5	6	7	13	19
1	DDT (50%)*, 1 lb. 2,4-dinitro-6-sec. butyl phenol. 1 oz. Monoethanolamine, 0.5 oz.	June 11	26.6	—	—	—	2.5	—	42.1
2	Same as Trial 1 but DDT omitted	June 30	42.1	2.2	—	—	—	7.9	—
3	DDT (50%), 1 lb. Dinitro-o-cyclohexylphenol (40%), 5 oz.	July 23	57.2	—	—	17.3	—	—	39.4
4	DDT (50%), 1 lb. Polyethylene polysulphide†, 1 pt. Dinitro-o-cyclohexylphenol, 1 oz. Monoethanolamine, 0.5 oz.	June 17	17.6	—	—	23.3	—	76.2	—
5	Dinitro-o-cyclohexylphenol, 1 oz. Monoethanolamine, 0.5 oz.	July 3	76.2	—	26.8	—	—	37.5	—

* Penco-WB-50.
† Good-Rite P.E.P.S.

On another block of McIntosh and Delicious apple trees, 40 per cent dinitro-o-cyclohexylphenol and the monoethanolamine salt of the technical compound were compared with Parathion and several other acaricides for

European red mite control. The materials were applied July 10 and July 14 without a spreading or wetting agent to plots consisting of 18 to 25 trees. Fifty per cent of these were sampled for mites immediately before spraying and 4 days, 15 to 19 days and 30 to 34 days after spraying. Results from the three materials are shown in Table 5.

TABLE 5.—COMPARISON OF 40 PER CENT DINITRO-O-CYCLOHEXYLPHENOL, THE MONOETHANOLAMINE SALT OF DINITRO-O-CYCLOHEXYLPHENOL AND PARATHION. AVERAGE NUMBER EUROPEAN RED MITE PER LEAF

Plot	Materials per 100 Imperial gallons	Before spray	4 days after	15-19 days after	30-34 days after
A	Dinitro-o-cyclohexylphenol 2 oz. Monoethanolamine 1 oz.	30.7	3.2	11.9	33.3
B	Dinitro-o-cyclohexylphenol (40%) 5 oz.	27.2	6.5	36.6	30.6
C	Parathion (15%)* 0.5 lb.	58.3	0.2	3.6	24.7

* Thiophos-3422.

DISCUSSION

Of the dinitrophenol derivatives most extensively examined for European red mite control in the Okanagan Valley of British Columbia, the monoethanolamine salt of 2,4-dinitro-o-cyclohexylphenol has been the most effective in respect of its initial kill and in its residual action. When used with DDT as a summer application at the rate of 2 ounces of the parent compound per 100 Imperial gallons of spray mixture this salt gave reasonably satisfactory control of European red mite both in experimental work and in the hands of many fruit-growers. At 1 ounce per 100 Imperial gallons it was not sufficiently toxic. In addition to the dinitrophenol derivatives mentioned in the tables (dinitro-o-cyclohexylphenol, monoethanolamine dinitro-o-cyclohexylphenolate, ammonium dinitro-o-cyclohexylphenolate, dicyclohexylamine dinitro-o-cyclohexylphenolate, dinitro-o-cresol, monoethanolamine dinitro-o-cresolate, and monoethanolamine dinitro-o-secondary butyl phenolate) the following acaricides were applied in field trials: summer oil, xanthone, hexaethyltetraphosphate, chlorinated camphene, hydroxypentamethylflavan, disodium ethylene bis bithiocarbamate, alkyl naphthyl ethers, rotenone-summer oil, water-soluble tobacco compounds. None of these was as satisfactory as monoethanolamine dinitro-o-cyclohexylphenolate. Dinitro-cyclohexylphenol used alone as "*DN-Dry Mix No. 1*" was apparently not quite as toxic to European red mite as its monoethanolamine salt.

Neither 3,5-dinitro-o-cresol nor its monoethanolamine salt had appreciable acaricidal value as summer sprays at feasible concentration. On the other hand, the monoethanolamine salt of 2,4-dinitro-6-secondary butyl phenol was promising when used at the rate of one ounce of the parent compound per 100 Imperial gallons of spray mixture. At double this concentration it may prove superior to an equal amount of the same salt of dinitro-o-cyclohexylphenol.

Among the acaricides employed simultaneously with DDT in four cover sprays in the experimental orchard the dicyclohexylamine salt of dinitro-o-cyclohexylphenol resulted in the heaviest European red mite damage. Used as the commercial formulation *DN-111*, at 0.5 pound per 100 Imperial gallons, that is, 1.6 ounces of the parent compound, it failed to give adequate control of European red mite. In fact, mite populations remained static after the first spray and quickly increased after two others. In grower trials under laboratory supervision from 0.75 pound to one pound of *DN-111* was added to a 50 per cent wettable DDT mixture in three or more cover sprays. The poor control of European red mite that followed suggests that this DDT formulation actually reduced the acaricidal value of the dicyclohexylamine salt. Cutright and Sutton (6) found that certain dinitrophenol derivatives in combination with DDT acted erratically in their performance against mites and in some instances caused fruit and foliage injury. Generally speaking, at least one pound of *DN-111* per 100 Imperial gallons has been necessary for European red mite control in British Columbia, and even then results have not been uniformly satisfactory. It is suspected that the type of the extender used with DDT may have some bearing on the matter.

On apples dinitro-o-cyclohexylphenol and its monoethanolamine salt have been used rather widely at effective dosages with little evidence of phytotoxicity under British Columbia conditions. In early cover sprays both materials on occasion have produced slight marginal burning of terminal leaves of water shoots. Although it is well known that the dinitrophenol derivatives in question cannot be applied to apple or pear foliage with impunity, there have been, in the Okanagan Valley, some surprising results from errors in concentration. The current summer strength recommendation for *DN-Dry Mix No. 1*, for example, is five ounces per 100 Imperial gallons; nevertheless, growers have used from one pound to even five pounds per 100 gallons without sign of foliage or fruit injury. Doubtless they were favoured by unusually cool weather since high temperatures appear to be conducive to damage from these preparations.

Pear foliage and fruits, particularly of the Anjou variety, seem to be more susceptible to injury from dinitrophenol derivatives than do apple foliage or fruits. In 1947 severe blackening of foliage on Anjou trees and pronounced lenticel injury on the fruit followed the application of a second cover spray of 50 per cent wettable DDT together with either the monoethanolamine salt or the dicyclohexylamine salt of dinitro-o-cyclohexylphenol. Bartlett pears were not so severely affected. Foliage injury was of no particular concern but fruit damage was still of commercial importance. During spraying and for two days thereafter maximum temperatures were 90°F., 90° F., and 83° F., and relative humidities were 27 per cent, 31 per cent and 41 per cent, respectively. It is presumed that unseasonably hot weather, coupled with the exceptional tenderness of the young pear foliage and fruits, brought about the injury. The use of dinitrophenol derivatives on pears is not recommended in the interior of British Columbia prior to the third cover spray.

It is interesting to note that the use of the monoethanolamine salt of dinitro-o-cylohexylphenol has resulted—at least in some cases—in increased size of fruit. According to packing-house officials, apples packed from orchards using this material were of exceptional size. That their impressions were probably correct is indicated by field experiments conducted by the Summerland Laboratory during 1941, 1942 and 1943. These experiments showed that fruits from McIntosh apple trees sprayed with sodium or ammonium dinitro-o-cresolate at the rate of 4 ounces of dinitro-o-cresol per 100 Imperial gallons in 5 codling moth cover sprays were approximately 20 per cent larger than those on adjacent trees that had received no dinitro-o-cresolate. Although instances of increased fruit size following the use of dinitrophenol derivatives may have been due in part to control of mites it seems more probable that it stemmed from chemical stimulation of the trees. It is hoped that this interesting manifestation will shortly be studied by the plant physiologists.

The experiments that have been discussed culminated in the official recommendation of the monoethanolamine salt of dinitro-o-cyclohexylphenol for the summer control of orchard mites in British Columbia during 1948. The recent improvements in acaricides has been so rapid, however, that this material may be supplanted within a year or two by one or more of the newer organic compounds presently under investigation.

SUMMARY

1. Since 1939 a number of dinitrophenol derivatives have been under investigation for orchard mite control in the Okanagan Valley of British Columbia. These derivatives included various formulations of the parent compounds 3, 5-dinitro-o-cresol, 2,4-dinitro-o-cyclohexylphenol and 2, 4-dinitro-o-secondary butyl phenol and some of their ammonium, monoethanolamine and dicyclohexylamine salts.

2. The monoethanolamine salt of dinitro-o-cyclohexylphenol at the rate of 2 ounces of the parent compound per 100 Imperial gallons of spray mixture has given most satisfactory mite control, taking effectiveness, cost and availability into consideration. When used with DDT in four codling moth cover sprays this compound gave better control of European red mite than did summer oil, xanthone, hexaethyltetraphosphate, chlorinated camphene, hydroxypentamethylflavan, disodium ethylene bis dithiocarbamate, alkyl naphthyl ethers, rotenone-oil and water soluble tobacco compounds.

3. The dicyclohexylamine salt of dinitro-o-cyclohexylphenol failed to give consistent commercial control of European red mite.

4. Dinitro-o-cyclohexylphenol with summer oil was fairly effective as a summer spray against both species of mites but considerable foliage injury followed its application. Used alone as the commercial formulation *DN-Dry Mix No. 1* at 5 ounces per 100 Imperial gallons this compound did not injure orchard foliage but apparently it was not quite as toxic to European red mite as its monoethanolamine salt.

5. Dinitro-o-cresol, with or without oil, at concentrations tolerated by apple trees was ineffective against both European red mite and Pacific mite, as also was its monoethanolamine salt.

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BOOK REVIEW

AN INTRODUCTION TO ORGANIC CHEMISTRY, by Roger J. Williams and Lewis F. Hatch, The University of Texas. Fifth Edition. 668 pp. 1948. D. Van Nostrand Company, Inc., Toronto. $6.50.

This textbook has been thoroughly revised and material included to keep pace with developments in the field of organic chemistry. The subject matter has been arranged in a logical manner with proper emphasis on industrial, medical and agricultural applications. The problems at the conclusion of each chapter assist the student in assessing his grasp of the subject and the inclusion in the text of biographical sketches of outstanding chemists adds interest to the book. Considerable care has been exercised in the selection of compounds to be represented by structural formulae. A sufficient number has been included to illustrate the many classes encountered in organic chemistry yet simplicity has not been sacrificed.

A complete index gives the book value as a reference as well as a text. Workers in agriculture might profitably consult it in order to gain a general picture of their problem before resorting to specialized material.

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